FIGURE 1
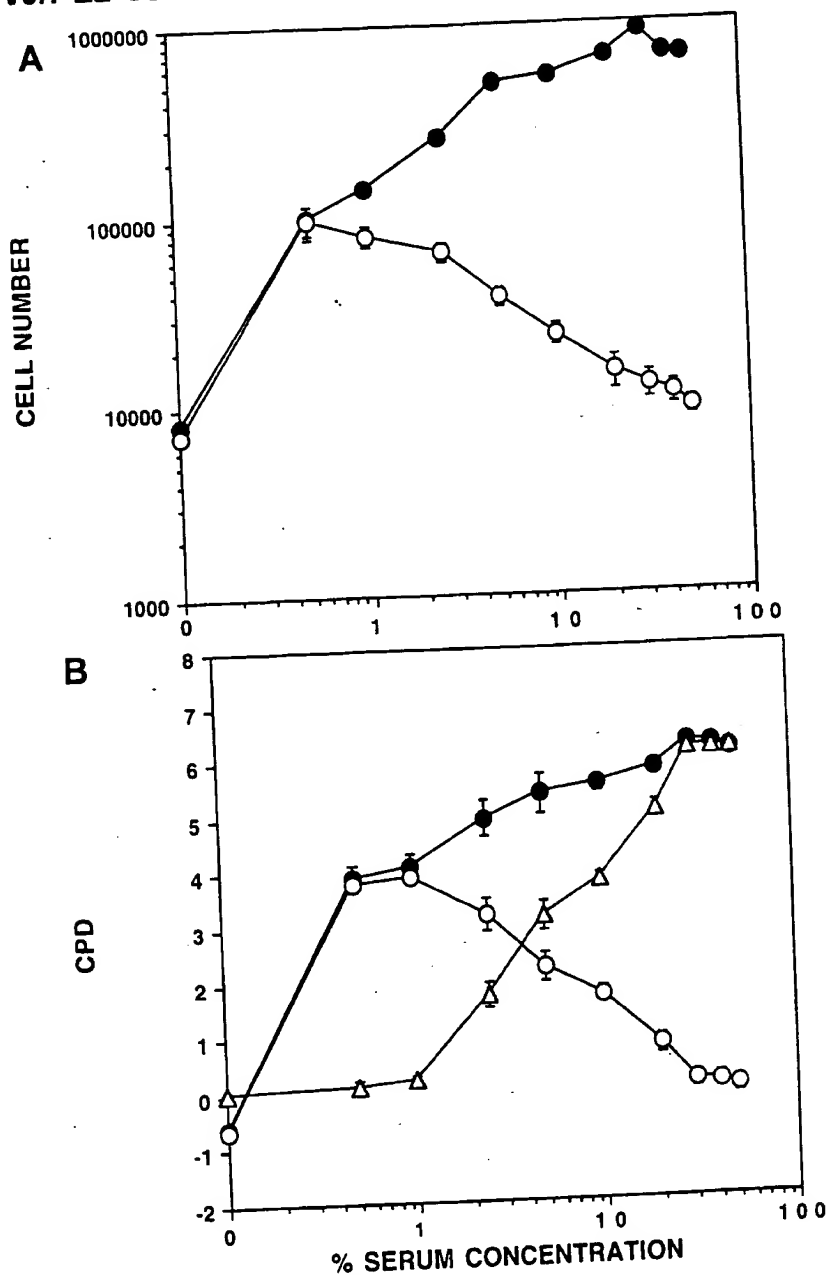
MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM


A: DATA EXPRESSED AS CELL NUMBER AFTER 7 DAYS
Growth with 1.0×10^{-8} M E_2 (closed circles) and
without hormone (open circles) in medium con-
taining the designated concentrations of serum.

B. DATA IN (A) EXPRESSED AS CPD
The symbols indicate the same conditions as (A)
except the open triangles show CPD differences
between growth in dishes with and without the
hormone (Difference = estrogenic effect on growth).

FIGURE 2

MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM WITH ESTROGENS ADDED AT VARIOUS TIMES AFTER SEEDING
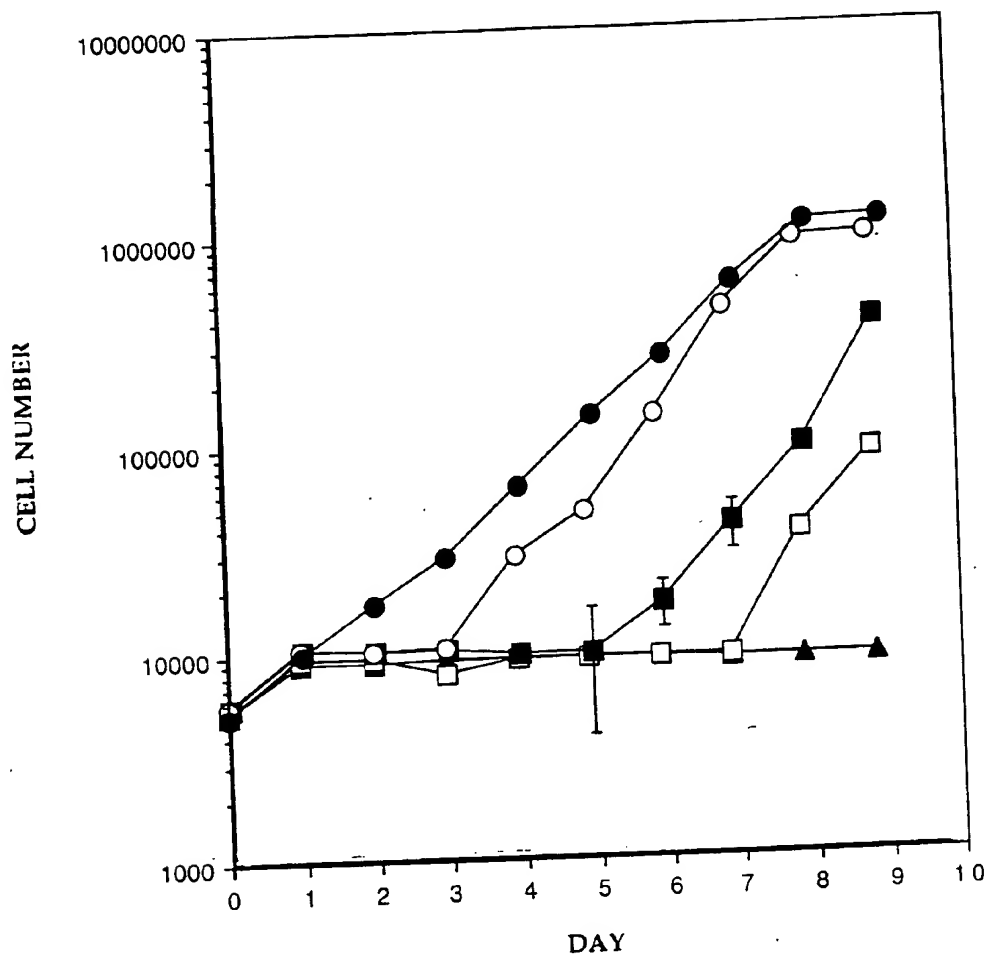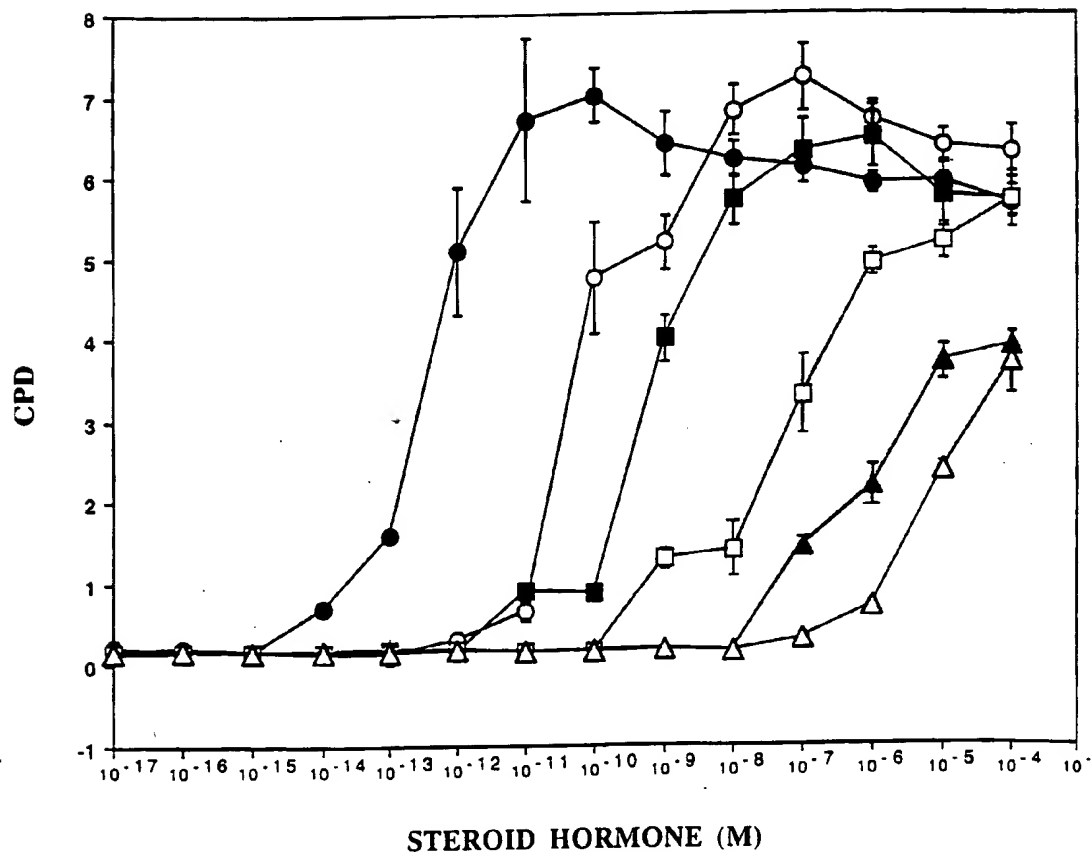

LEGEND:

Control growth in the absence of exogenous estrogen is shown by (triangles). In other dishes, 1.0×10^{-8} M E_2 was added at the beginning of the experiment (closed circles), after 48 h (open circles), after 96 h (closed squares), or after 144 h (open squares).

FIGURE 3

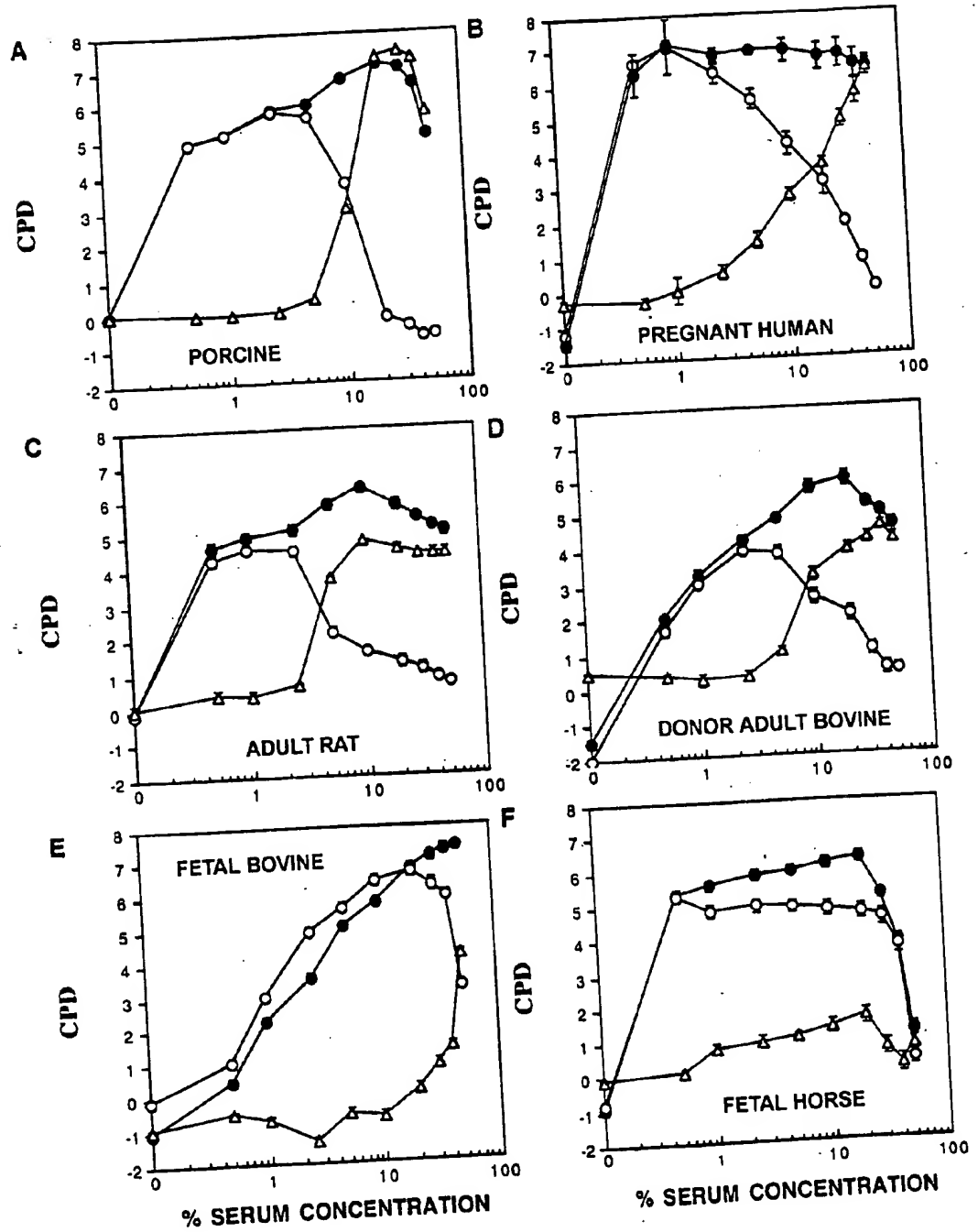## STEROID HORMONE DOSE RESPONSE EFFECTS WITH MTW9/PL2 CELLS IN 50% CDE - HORSE SERUM

STEROID HORMONE (M)

LEGEND:

Closed circles = E_2
Open circles = E_1
Closed squares = E_3
Open squares = Progesterone
Closed triangles = DHT
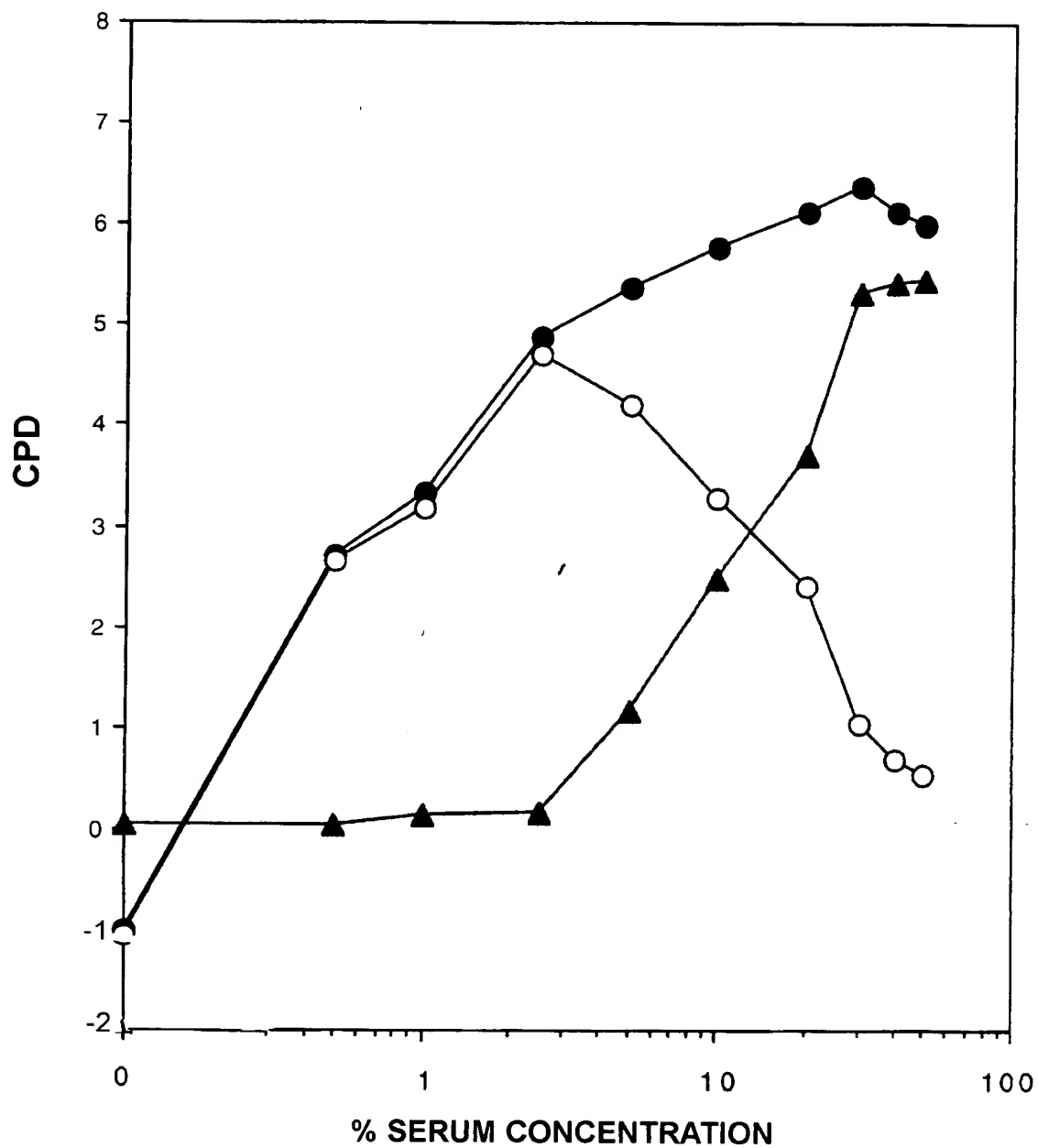Open triangles = T

FIGURE 4

MTW9PL2 CELL GROWTH IN CDE SERUM
FROM DIFFERENT SPECIES

LEGEND: Open circles = $-E_2$
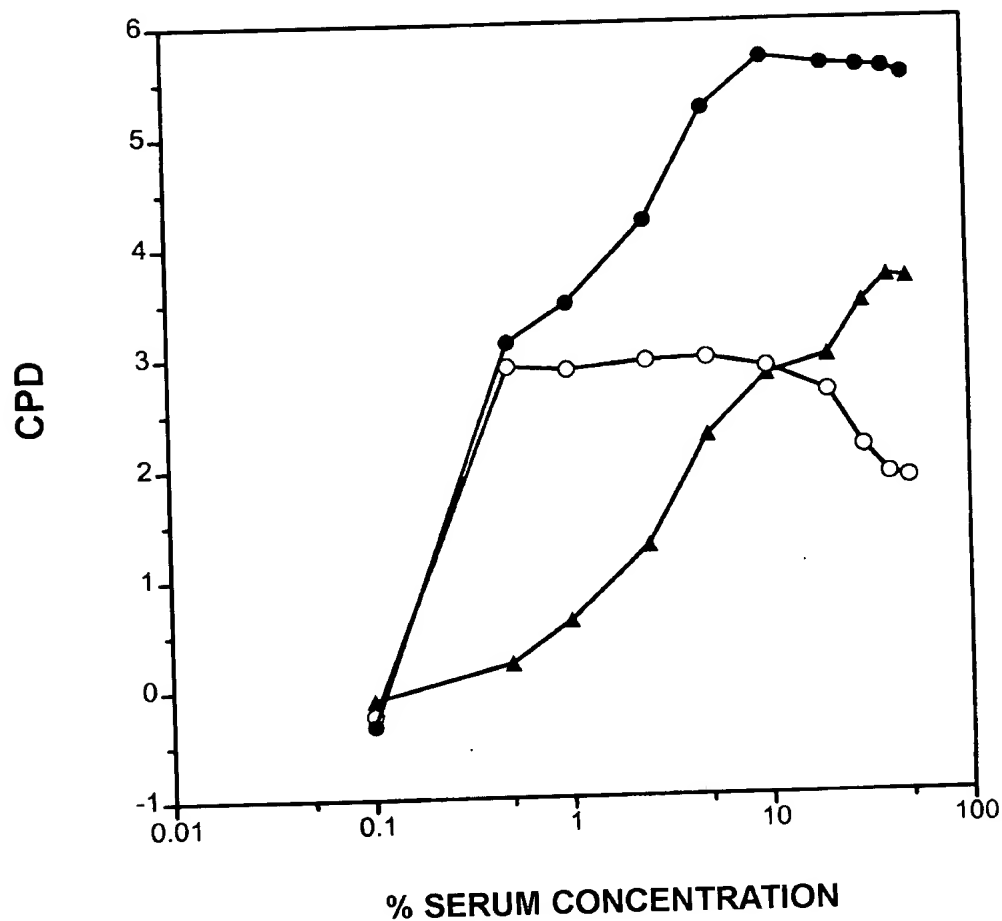Closed circles = $+E_2$
Open triangles = Estrogenic effect

FIGURE 5

CDE HORSE SERUM TITRATION
GH4C1 CELLS

LEGEND: ──●── = + E_2

──○── = − E_2

──▲── = Estrogenic effect

FIGURE 6

ZR-75-1 CELLS IN CDE - HORSE SERUM $\pm$ 10 nM E_2
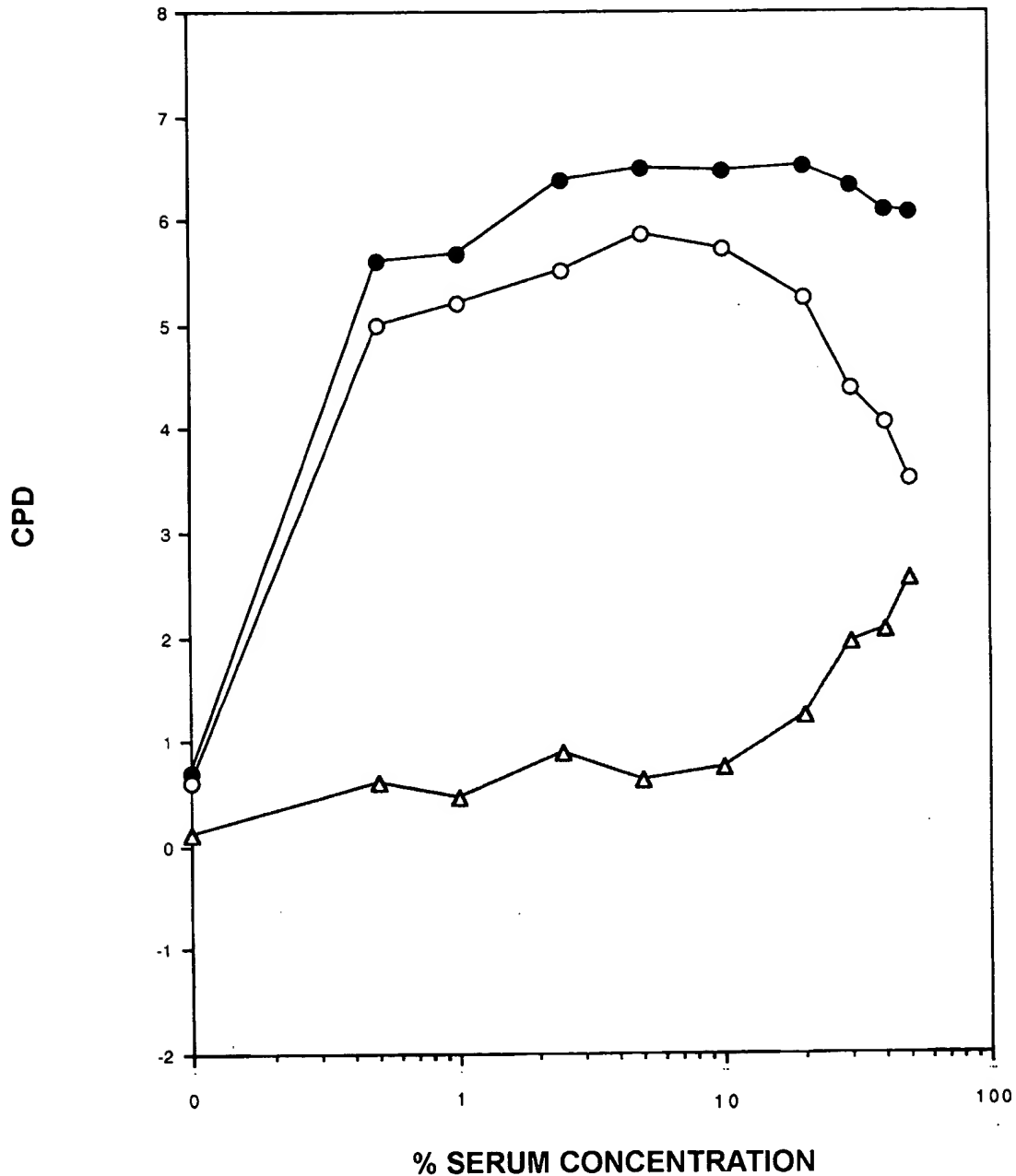

LEGEND:

Closed circles = $+E_2$
Open circles = $-E_2$
Closed triangles = Estrogenic effect

FIGURE 7

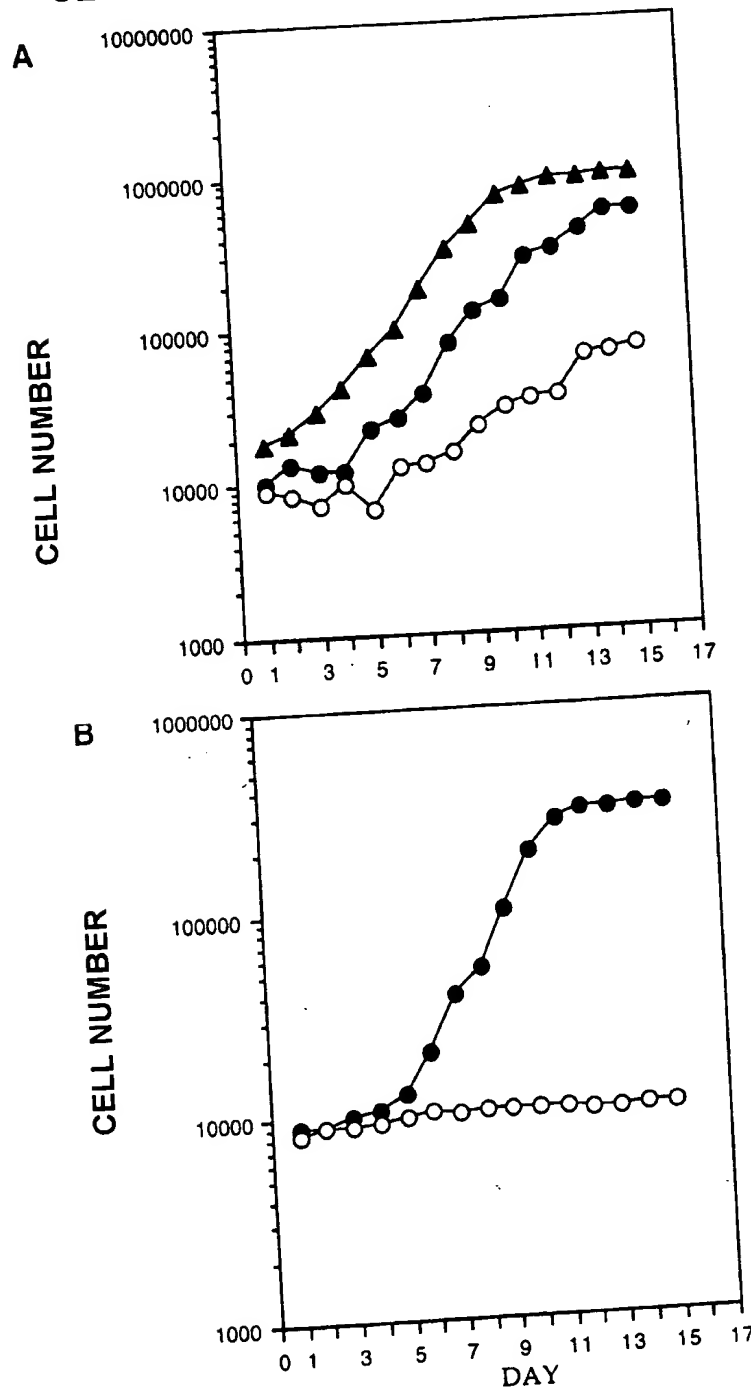## MCF7A CELL GROWTH IN CDE - HORSE SERUM $\pm E_2$

LEGEND:

Closed circles = $+E_2$
Open circles = $-E_2$
Closed triangles = Estrogenic effect

FIGURE 8

## GROWTH KINETICS OF T47D HUMAN BREAST CANCER CELLS IN CDE - HORSE SERUM ±10 nM E$_2$

(A) The growth of the cells in medium with 20% (v/v) serum with 10 nM E$_2$ (closed circles) and without the steroid (open circles). As comparison, growth is shown in medium containing 10% (v/v) FBS (triangles).

(B) T47D cell growth kinetics in medium with 50% (v/v) serum with E$_2$ (closed circles) and without the steroid (open circles).
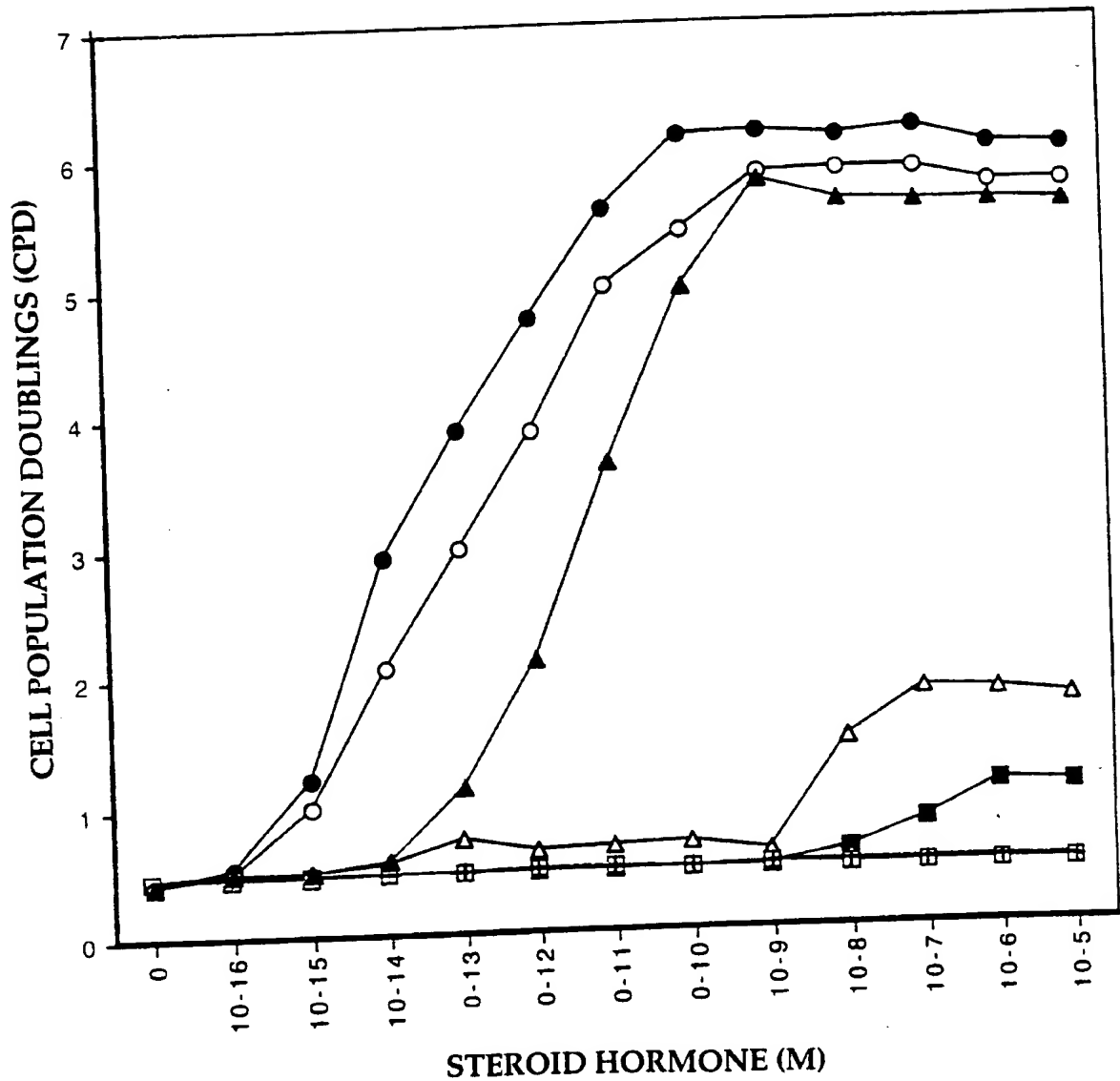
FIGURE 9

GROWTH OF HUMAN & RODENT CELL LINES
IN 50% CDE - HORSE SERUM $\pm E_2$ (10 nM)

LEGEND: Closed circles = Medium with 10 nM E_2
Open circles = Medium without E_2
Triangles = Estrogenic effect
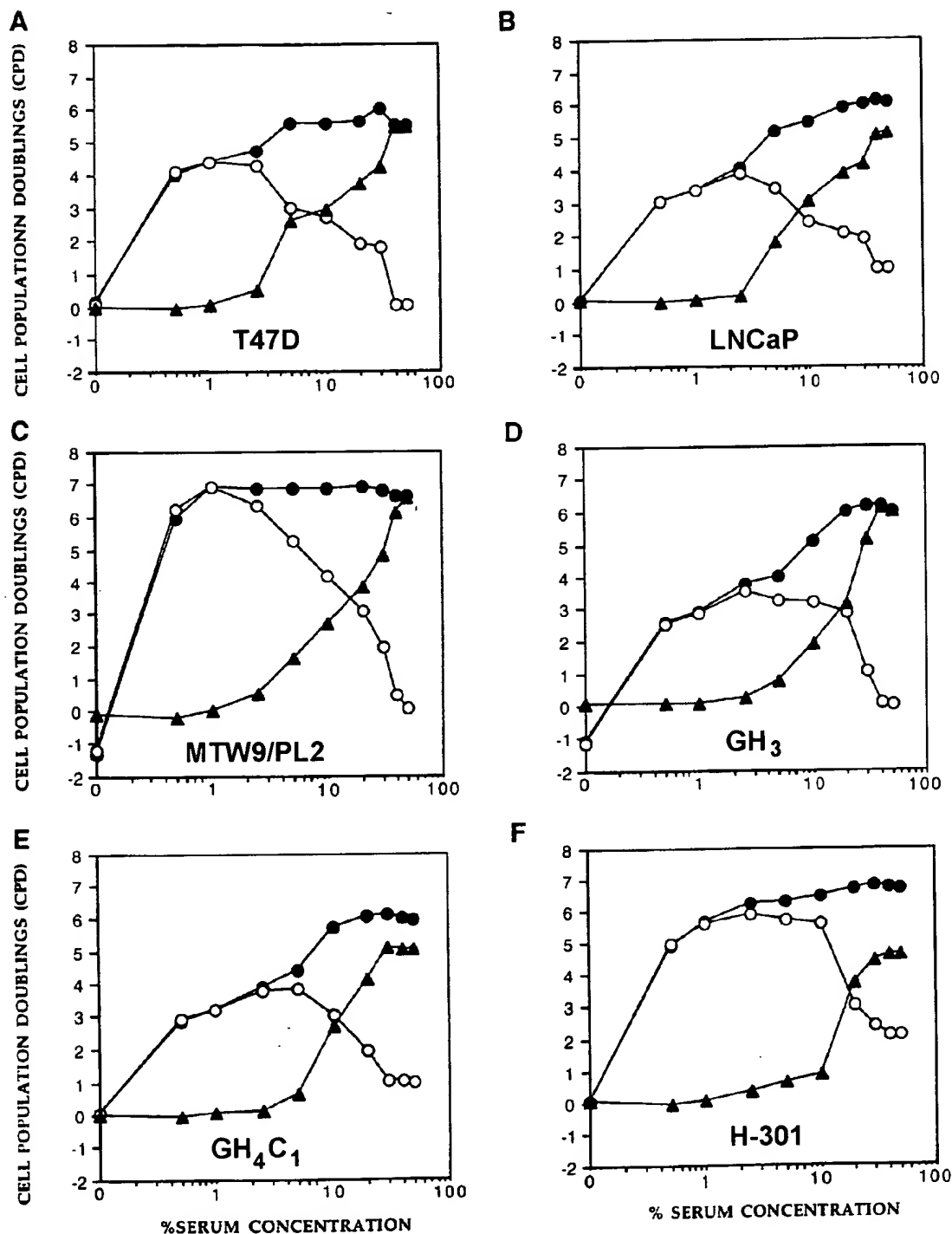
FIGURE 10

DOSE RESPONSE OF STEROID HORMONES
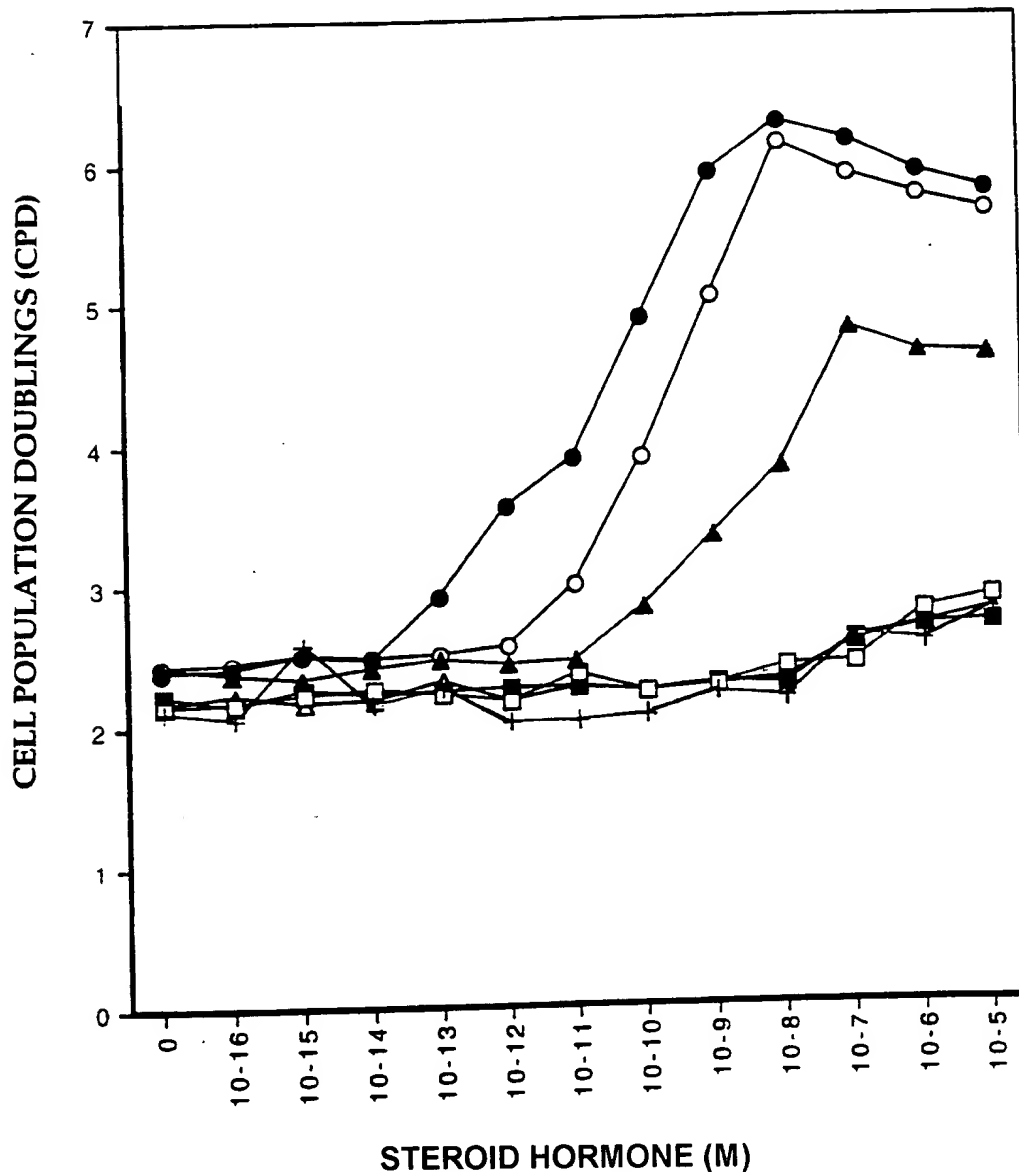## WITH T47D CELLS IN 50% CDE - HORSE SERUM

LEGEND:

Growth after 14 days is shown in response to:
Closed circles = E_2
Open circles = E_1
Closed triangl s = E_3
Open triangles = DHT
Closed squares = Testosterone
Open squares = Progesteron
Crosses = Cortisol

FIGURE 11

DOSE RESPONSE OF STEROID HORMONES
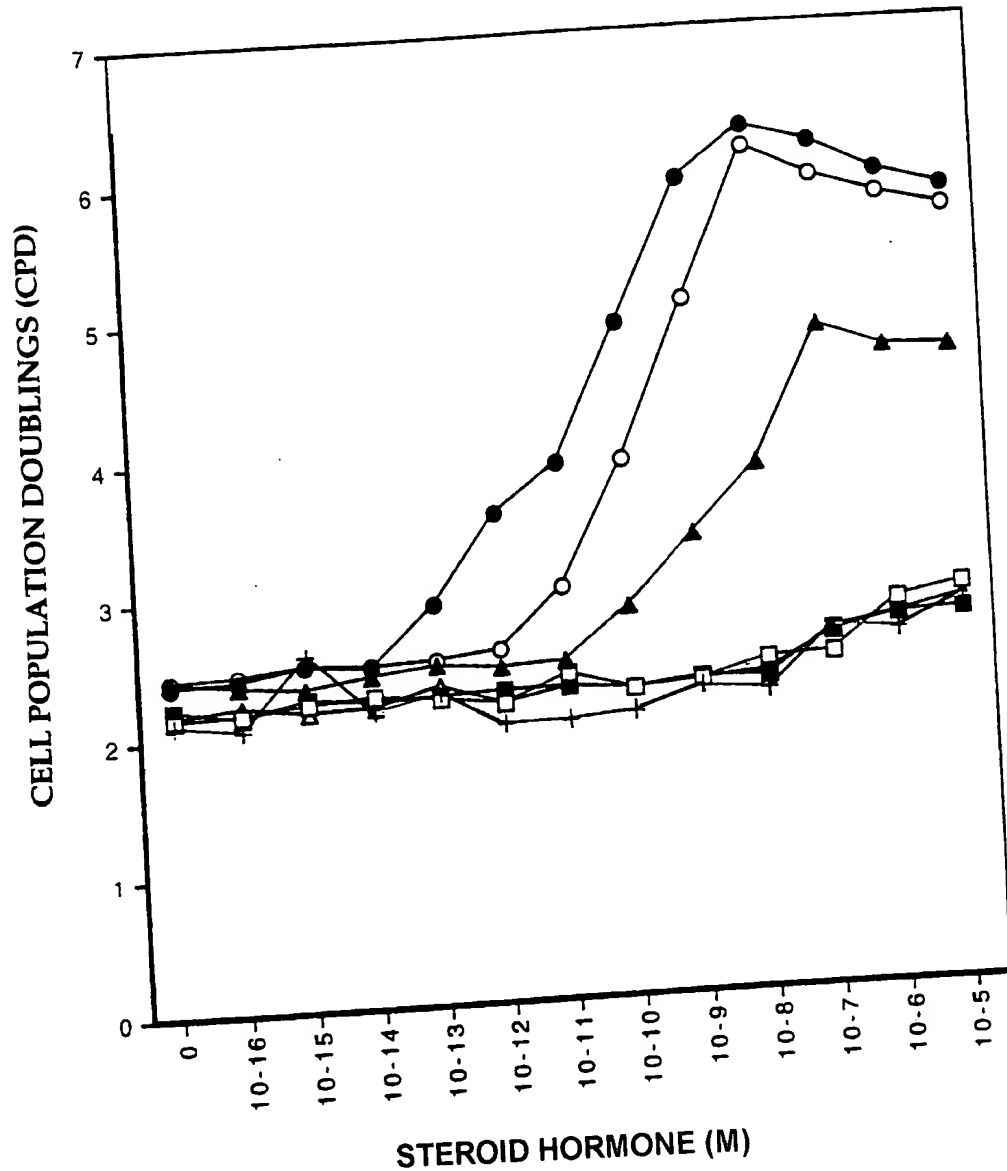## WITH H-301 CELLS IN 50% CDE - HORSE SERUM

STEROID HORMONE (M)

LEGEND:

Growth after 9 days is shown in response to:
Closed circles = E_2
Open circles = E_1
Closed triangles = E_3
Open triangles = DHT
Closed squares = Testosterone
Open squares = Progesterone
Crosses = Cortisol

FIGURE 12

DOSE RESPONSE OF STEROID HORMONES
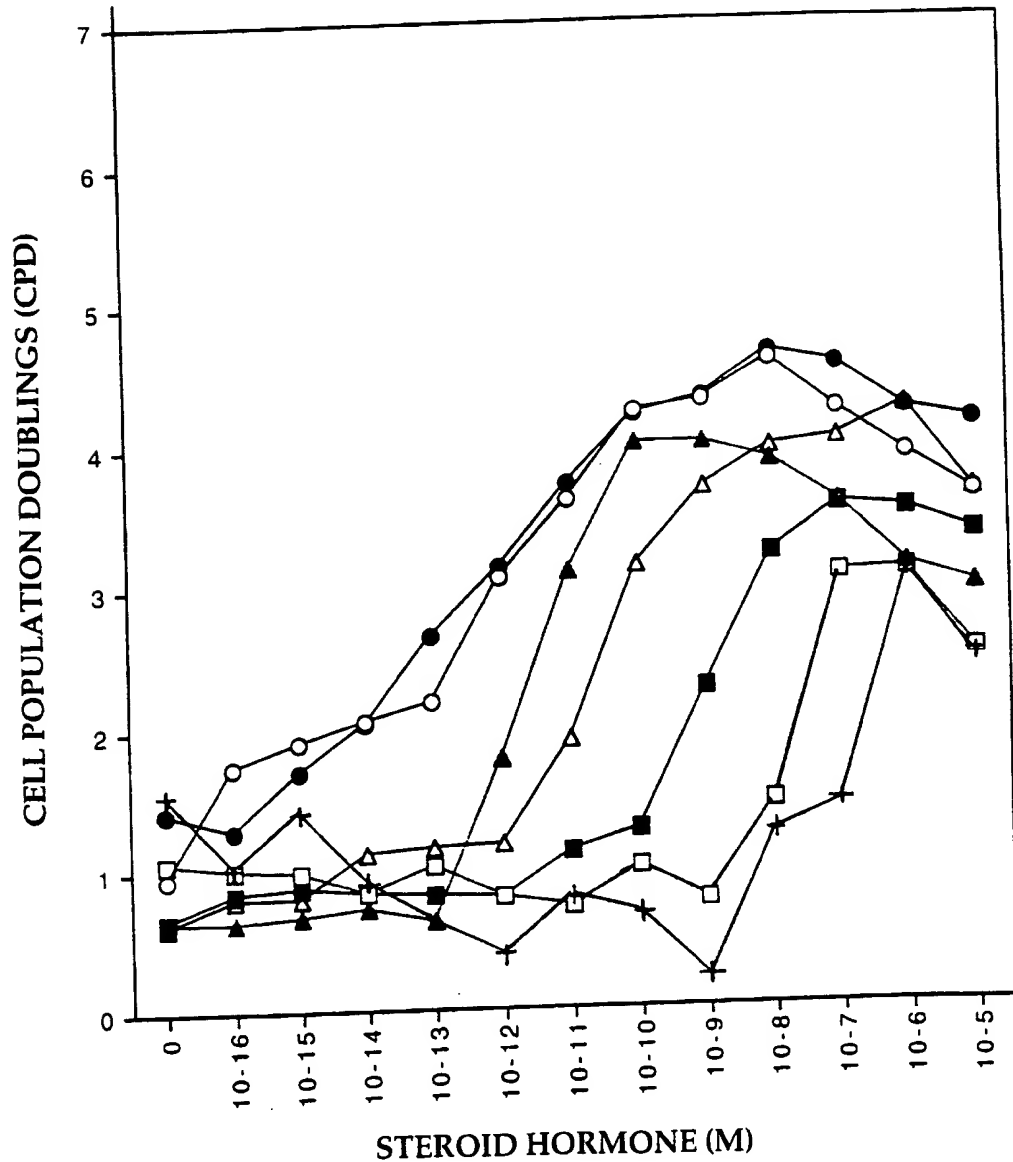## WITH H-301 CELLS IN 50% CDE - HORSE SERUM

LEGEND:

Growth after 9 days is shown in response to:
Closed circles = E_2
Open circles = E_1
Closed triangles = E_3
Open triangles = DHT
Closed squares = Testosterone
Open squares = Progesterone
Crosses = Cortisol

FIGURE 13

DOSE RESPONSE OF STEROID HORMONES
WITH LNCaP CELLS IN 50% CDE - HORSE SERUM

LEGEND:

Growth after 14 days is shown in response to:
Closed circles = E_2
Open triangles = E_1
Open squares = E_3
Open circles = DHT
Closed triangles = Testosterone
Closed squares = Progesterone
Crosses = Cortisol

FIGURE 14

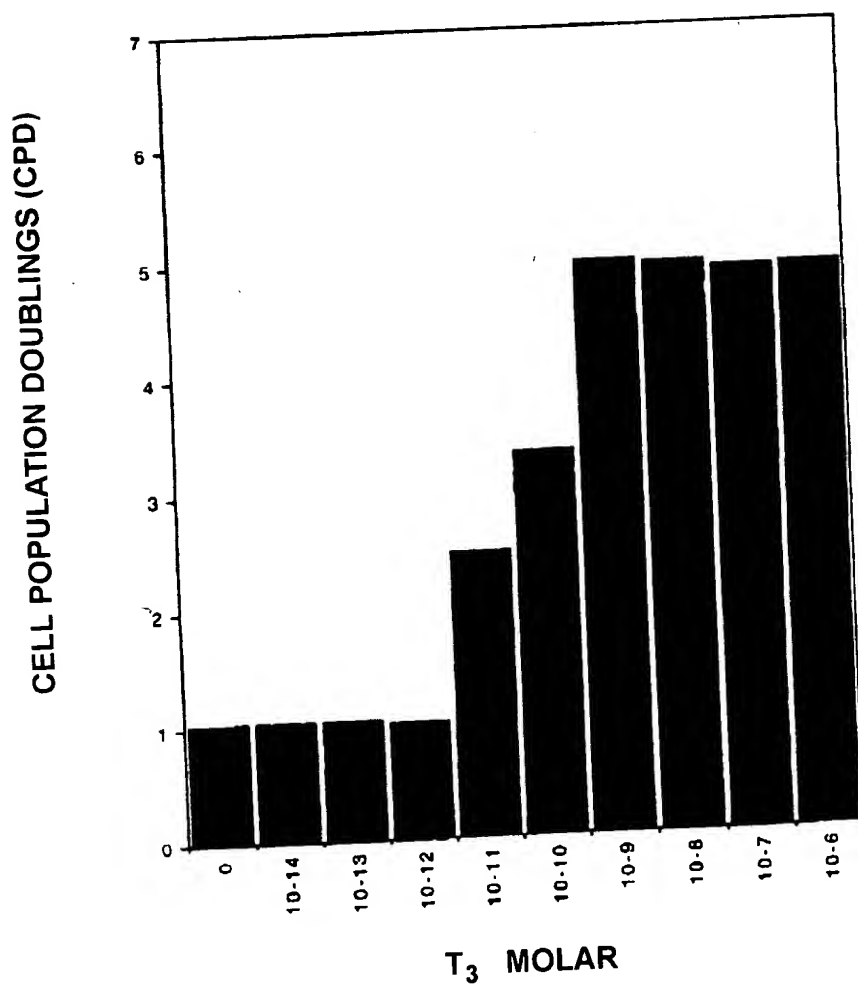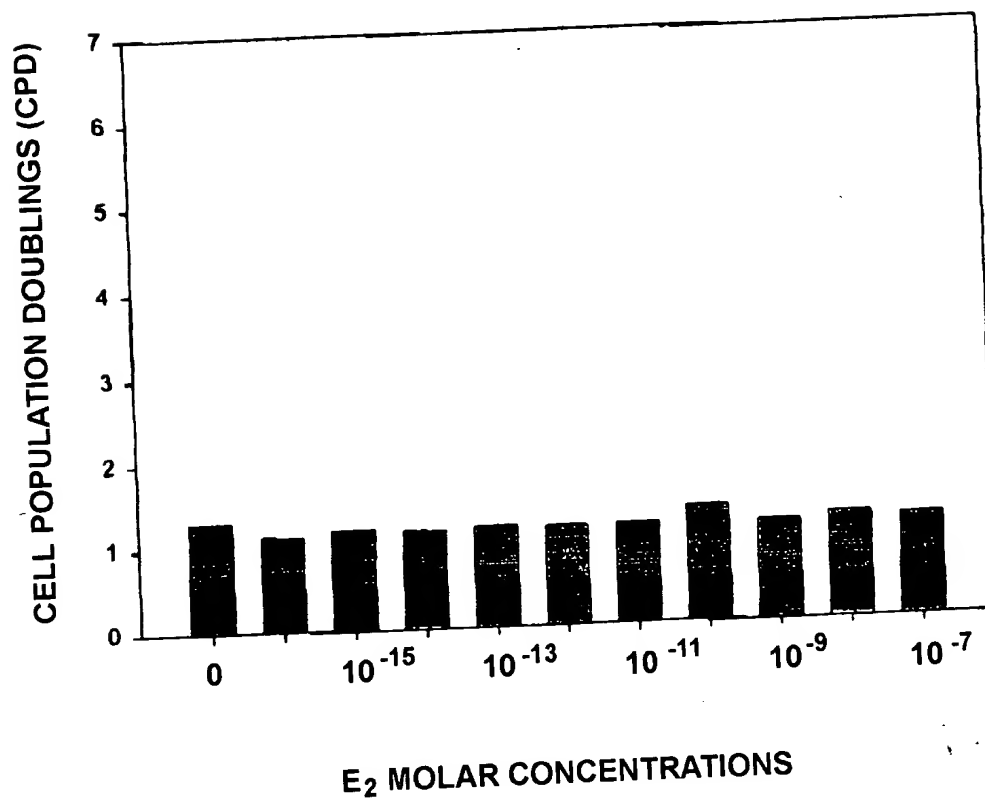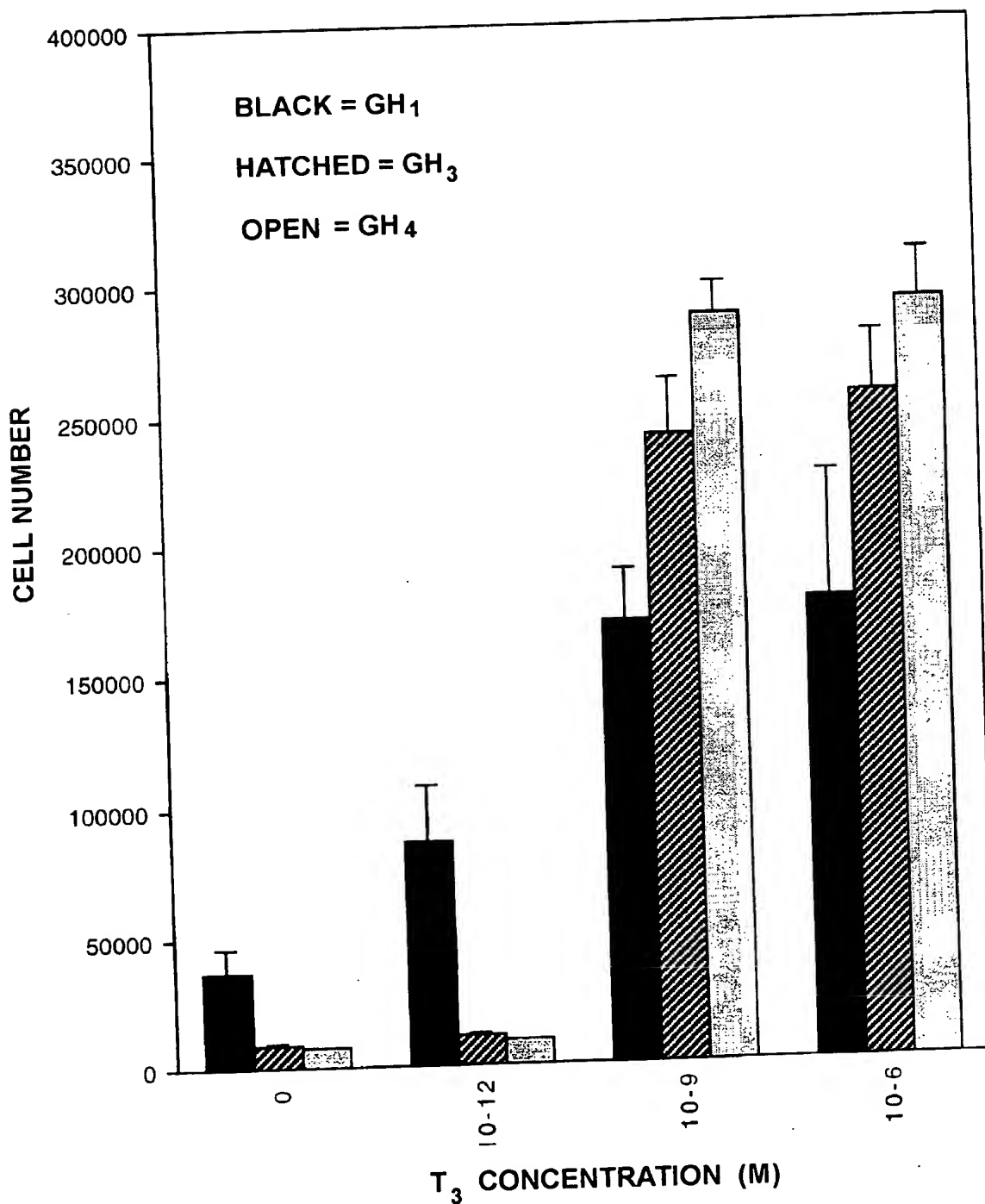## T_3 TITRATION OF GH_3 CELLS GROWN
IN SERUM - FREE MEDIUM (PCM)

FIGURE 15

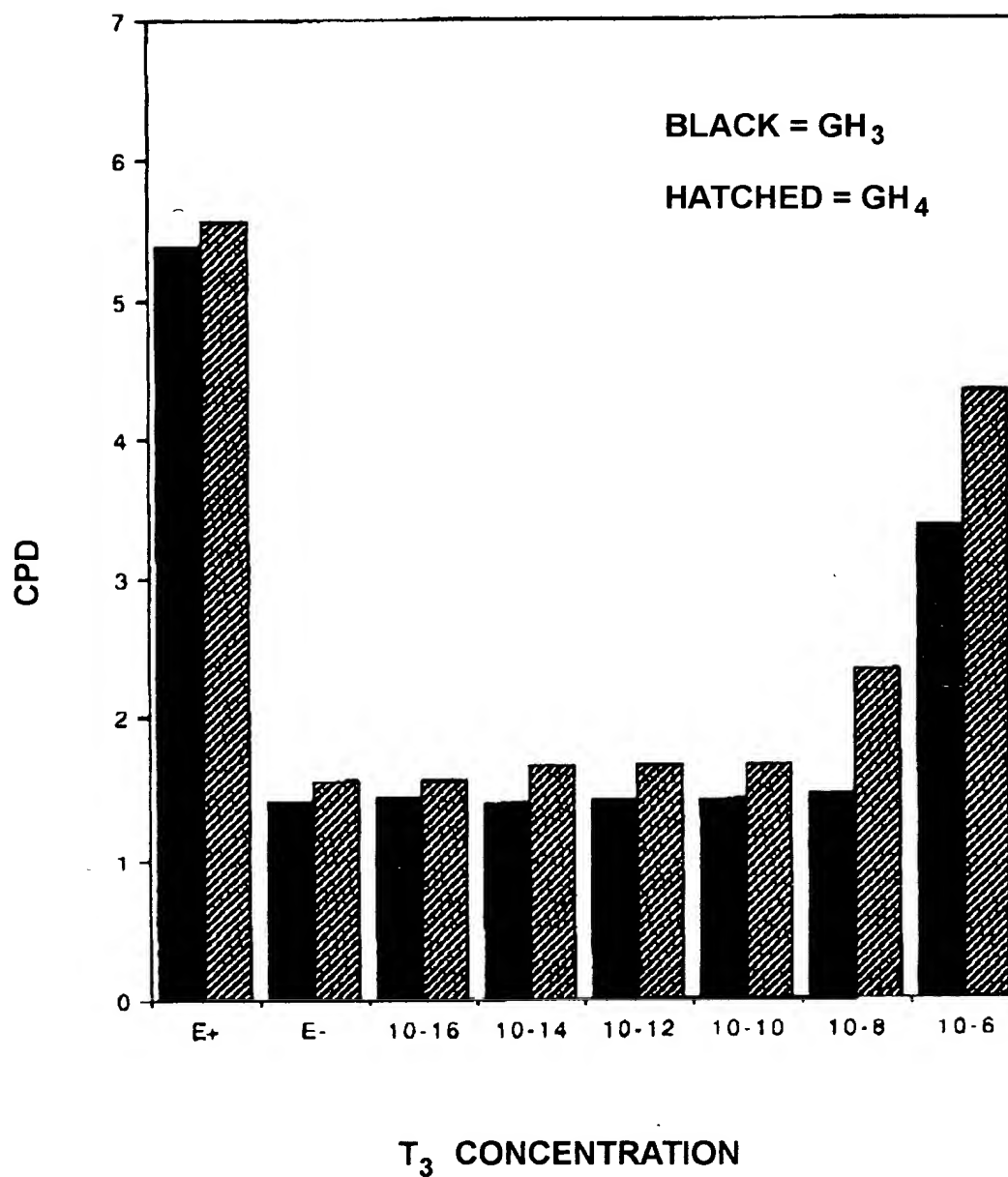## E_2 TITRATION OF GH_3 CELLS GROWN IN SERUM-FREE MEDIUM MINUS T_3

FIGURE 16

EFFECT OF T_3 ON GH CELL LINES:
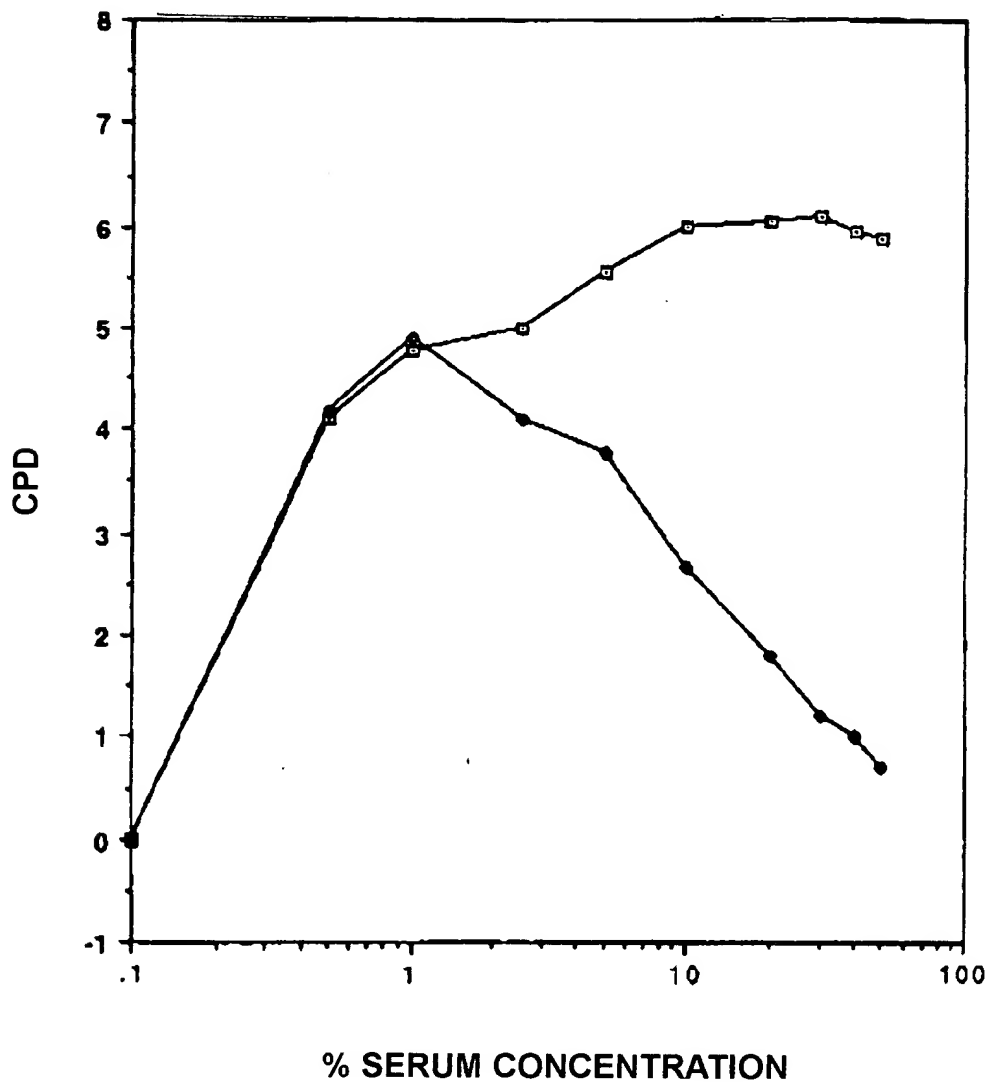GROWTH IN 2.5% CDE - HORSE SERUM WITH NO E_2

FIGURE 17

EFFECT OF T_3 ON PITUITARY CELL LINES
INCUBATED IN 50% CDE - HORSE SERUM

BLACK = GH_3

HATCHED = GH_4

CPD

E+ E- 10-16 10-14 10-12 10-10 10-8 10-6

T_3 CONCENTRATION

FIGURE 18

EFFECT OF XAD-4 RESIN TREATED HORSE SERUM
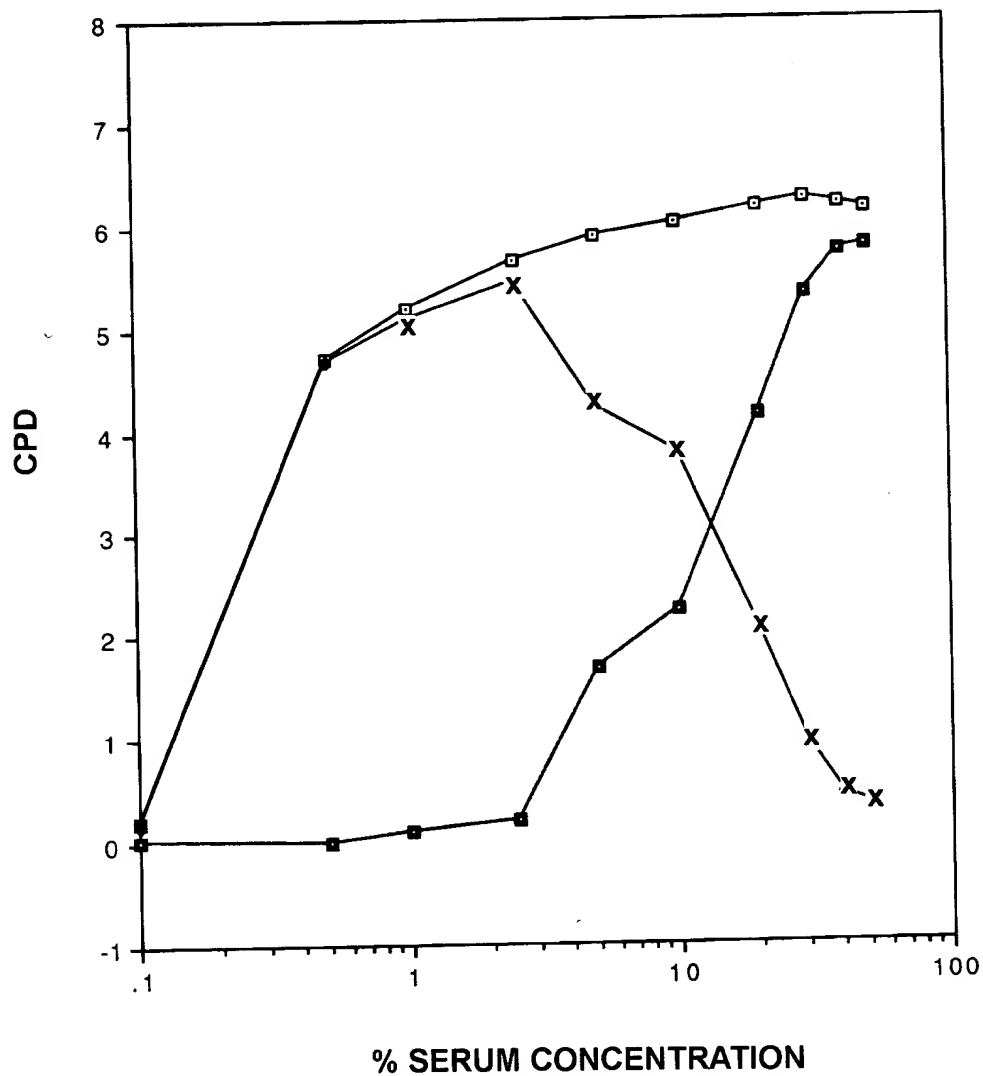## ON MTW9/PL2 CELL GROWTH $\pm E_2$

LEGEND:

Open squar s = $+ E_2$

Closed squares = $- E_2$

FIGURE 19

EFFECT OF XAD-4 RESIN TREATED HORSE SERUM
ON T47D CELL GROWTH $\pm$ E$_2$

LEGEND:
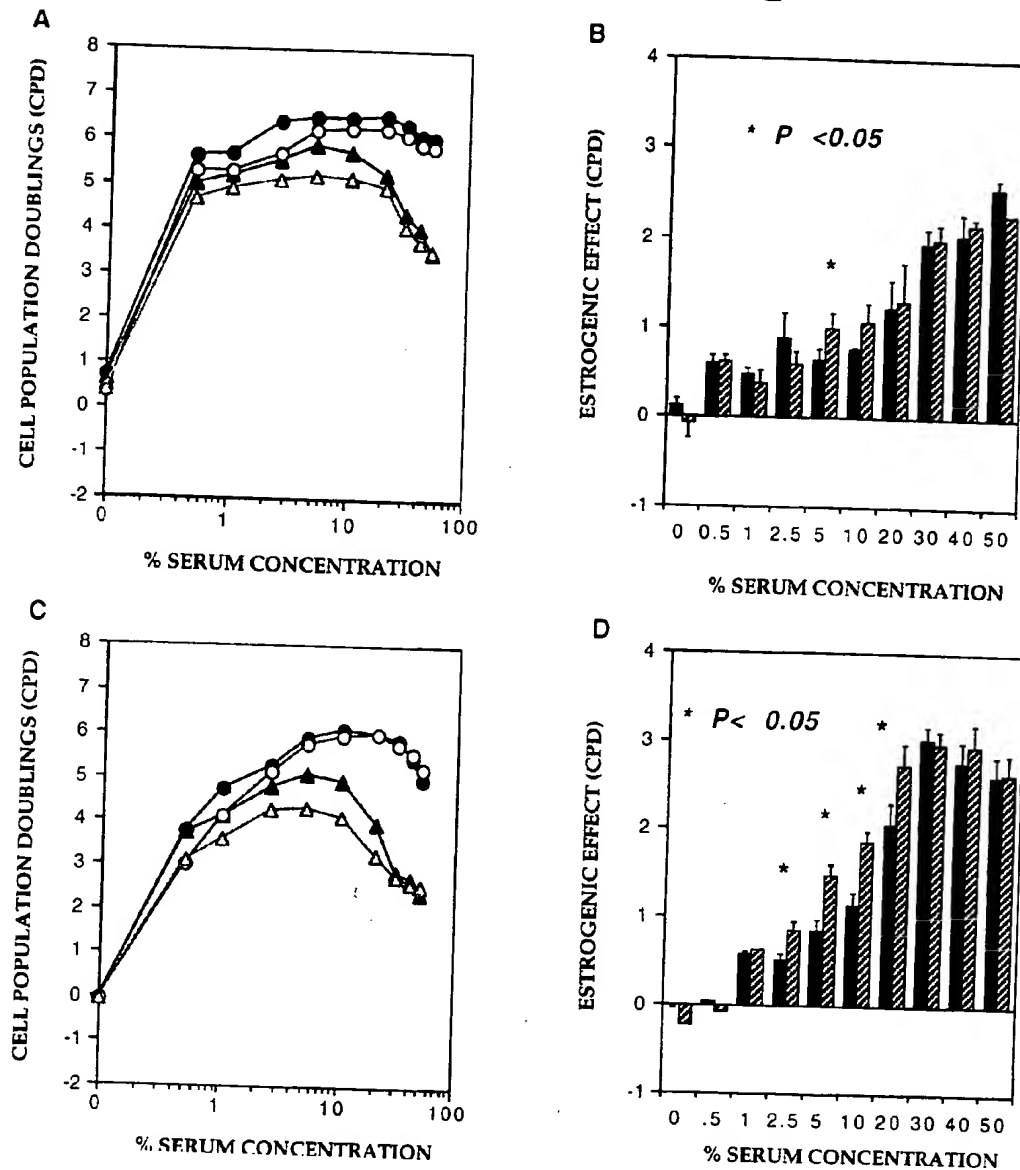
Open squares = + E$_2$

XXX = - E$_2$

Closed squares = Estrogenic effect

FIGURE 20

MCF-7 CELL GROWTH IN CDE - HORSE SERUM ± PHENOL RED AND ± E₂
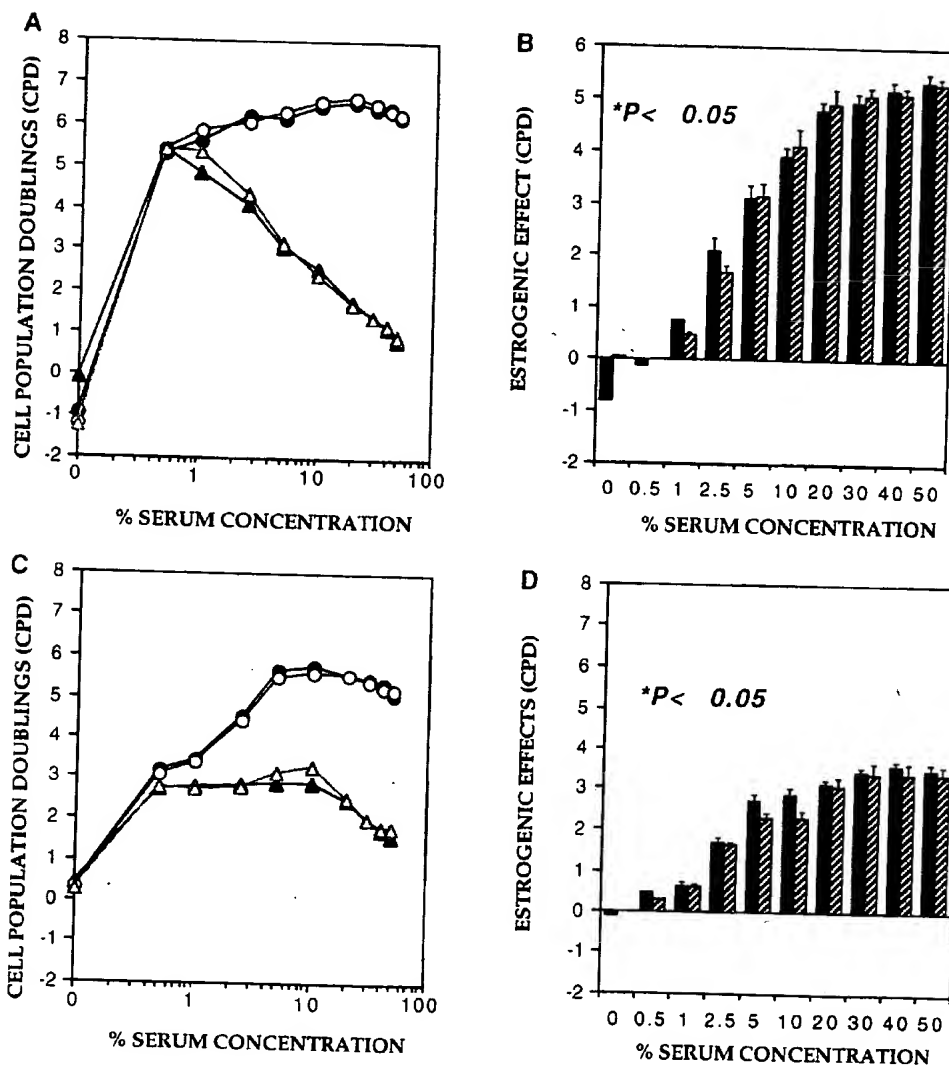

LEGEND:

(A) MCF-7A cell growth in phenol red containing medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(B) Estrogenic effects with MCF-7A cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E_2 minus the CPD in medium without added E_2.

(C) MCF-7K cell growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(D) Estrogenic effects with MCF-7K cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 21

T47D AND ZR-75-1 CELL GROWTH
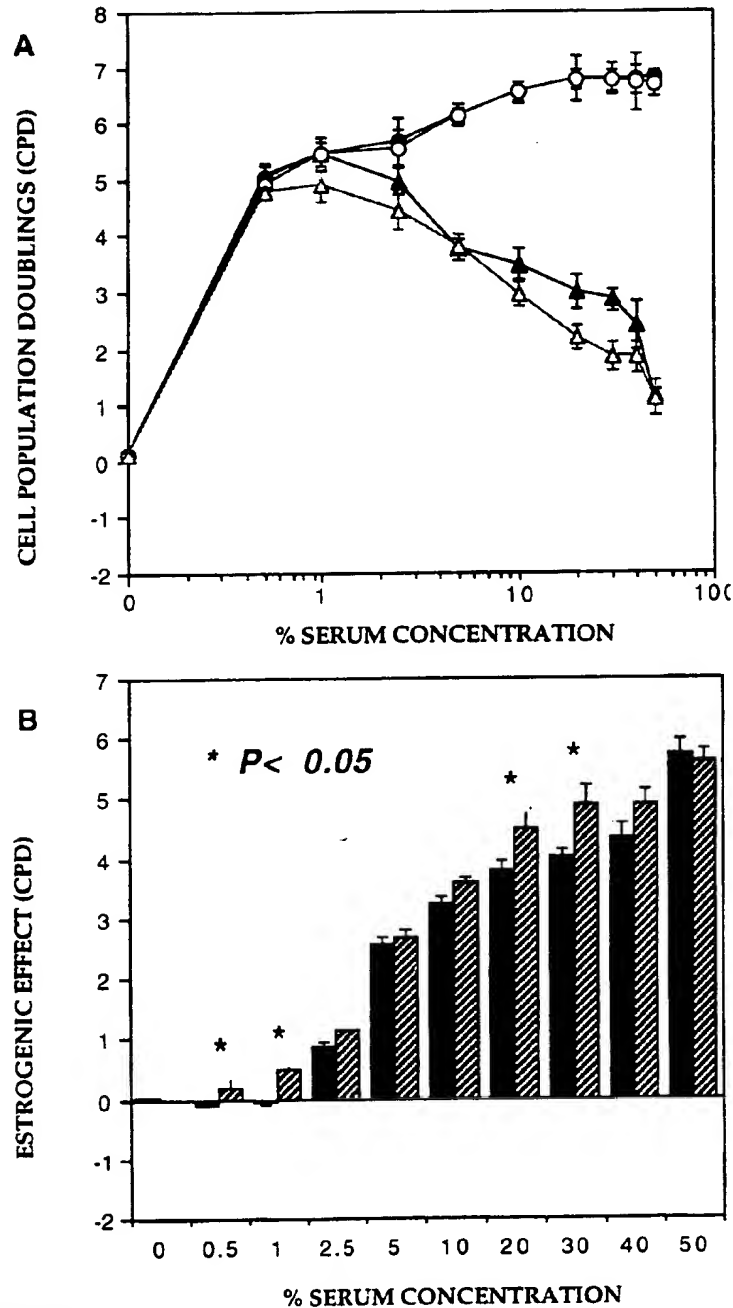## IN CDE-HS ± PHENOL RED AND ± E_2

LEGEND:

(A) T47D cell growth in phenol red containing medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(B) Estrogenic effects with T47D cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E_2 minus the CPD in medium without added E_2 .

(C) ZR-75-1 cell growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(D) Estrogenic effects with ZR-75-1 cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 22

MTW9/PL2 CELL GROWTH IN CDE - HORSE SERUM
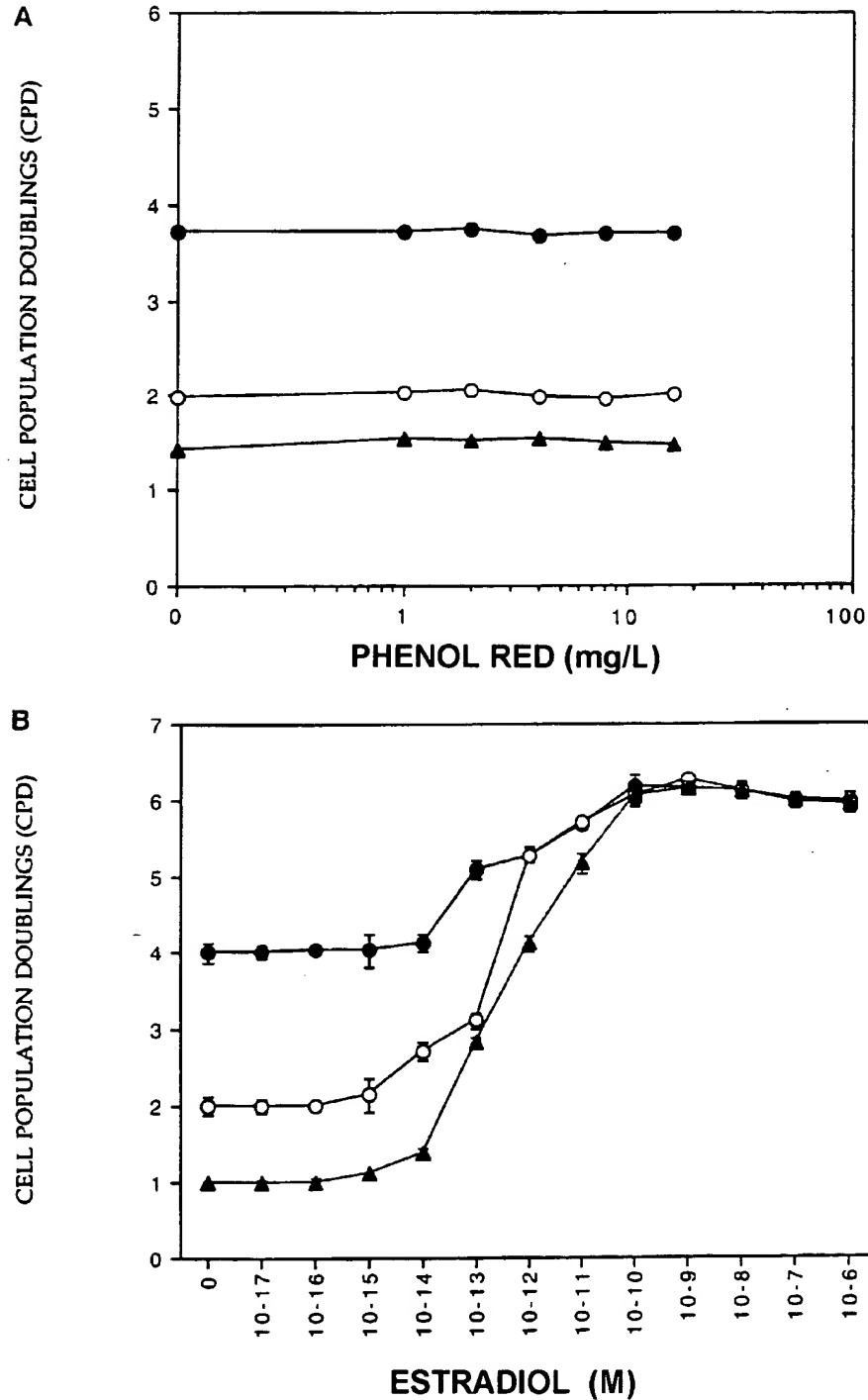## ± PHENOL RED AND ± E_2

LEGEND:

(A) MTW9/PL2 growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(B) Estrogenic effects with MTW9/PL2 cells in medium with phenol red (solid bars) and without (shaded bars) were calculated from (A).
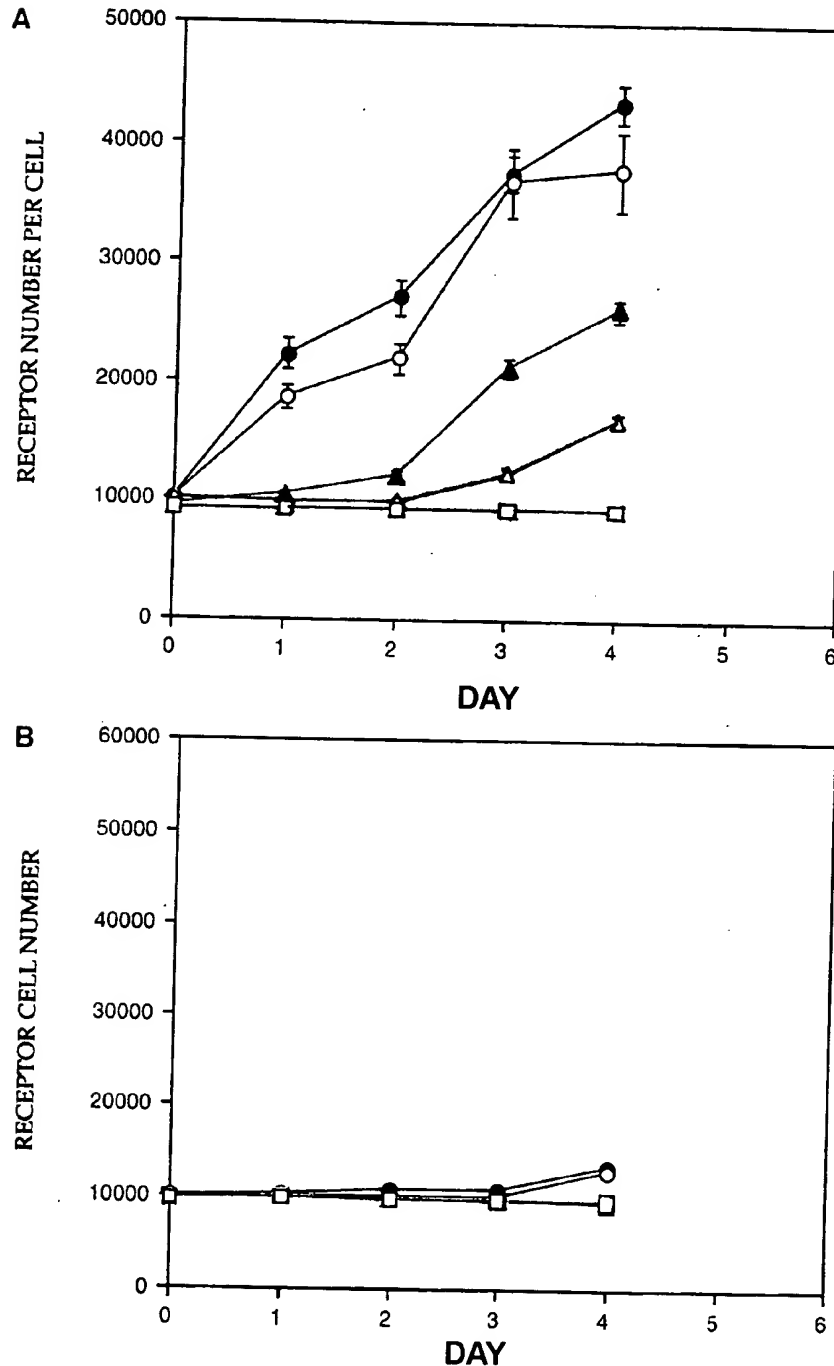
FIGURE 23

DOSE RESPONSE TO PHENOL RED AND E_2 IN THREE CELL LINES

LEGEND: The growth of the MCF-7A (closed circles), MTW9/PL2 (open circles) and T47D (closed triangles) cell lines was assessed at 14, 7, and 12 days.

FIGURE 24

## PROGESTERONE RECEPTOR INDUCTION IN T47D CELLS BY PHENOL RED AND E_2

A

B

LEGEND:

(A) The effects of E_2 at 1.0 x 10^{-8}M (closed circles), 1.0 x 10^{-10} M (open circles), 1.0 x 10^{-12}M (closed triangles), 1.0 x 10^{-14} M (open triangles) and the control without added E_2 (open squares).

(B) The effects of phenol red at 16 mg/L (closed circles), 8mg/L (open circles), 4 mg/L (closed triangles), 2 mg/L (open triangles), and the control without phenol red (open squares).

FIGURE 25

## EFFECT OF TGF-beta1 ON THE GROWTH OF BREAST/MAMMARY ORIGIN CELL LINES
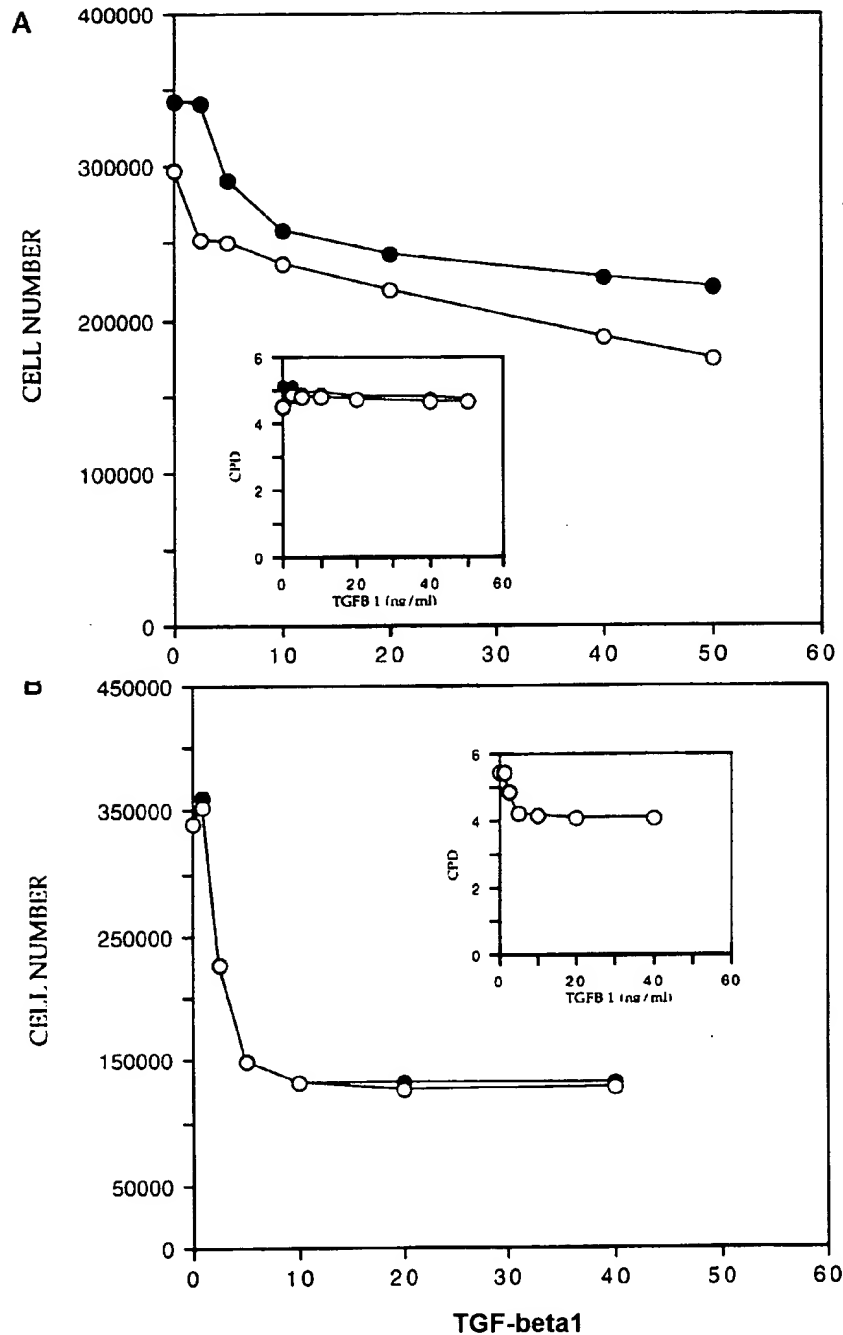
LEGEND:

(A) The effect of the transforming growth inhibitor on human breast MCF-7K cell growth as measured after 12 d either with 10 nM E_2 (closed circles) or without the hormone (open circles). The insert shows conversion of the cell number results to CPD.

(B) The same experiment with rat mammary MTW9/PL2 cells after 9 d growth.

FIGURE 26

## EFFECT OF TGF-beta1 ON THE GROWTH OF CELL LINES FROM BOTH HUMAN AND RODENT TUMORS
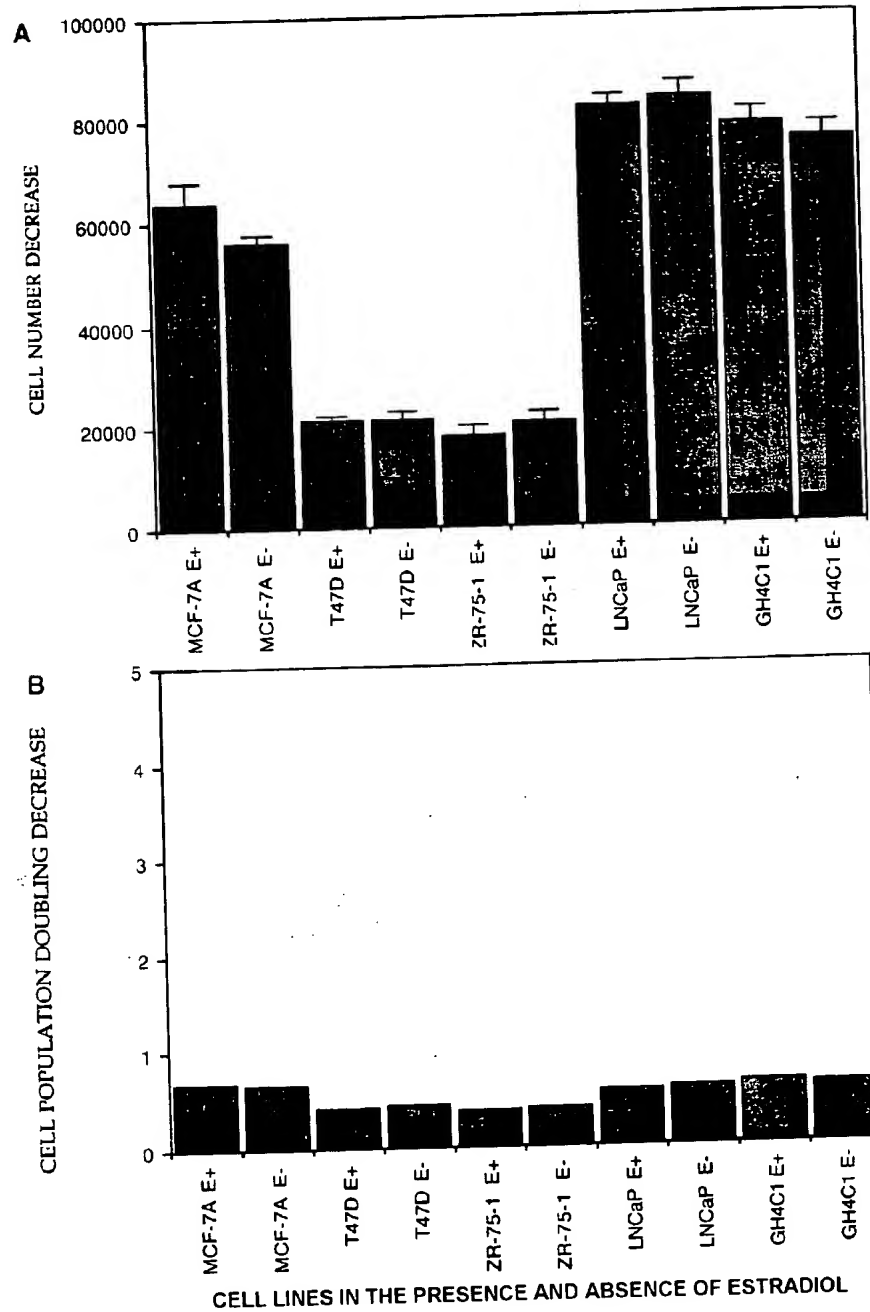
In these studies, TGF-beta1 was added at 40 ng/ml. Estradiol (± E) indicates either no added E$_2$ or the steroid at 10 nM.

(A) The effect of TGF-beta1 on five cell lines after 10-14 d growth in medium ± E$_2$. Th   results are expressed as cell number decreases caused by TGF-beta1.

(B) The CPD d creases caused by TGF-beta1 ± E$_2$ with each of the cell lines shown in (A).
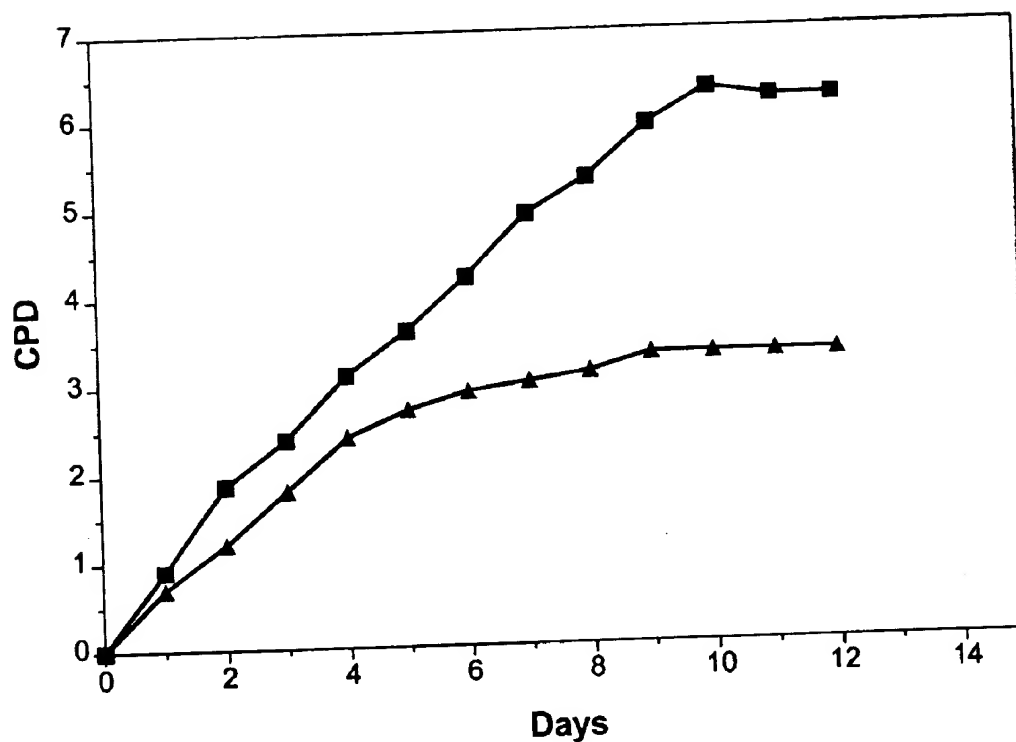
FIGURE 27

EFFECT OF EGF AND TGF-alpha ON THE GROWTH OF HUMAN BREAST CANCER CELLS
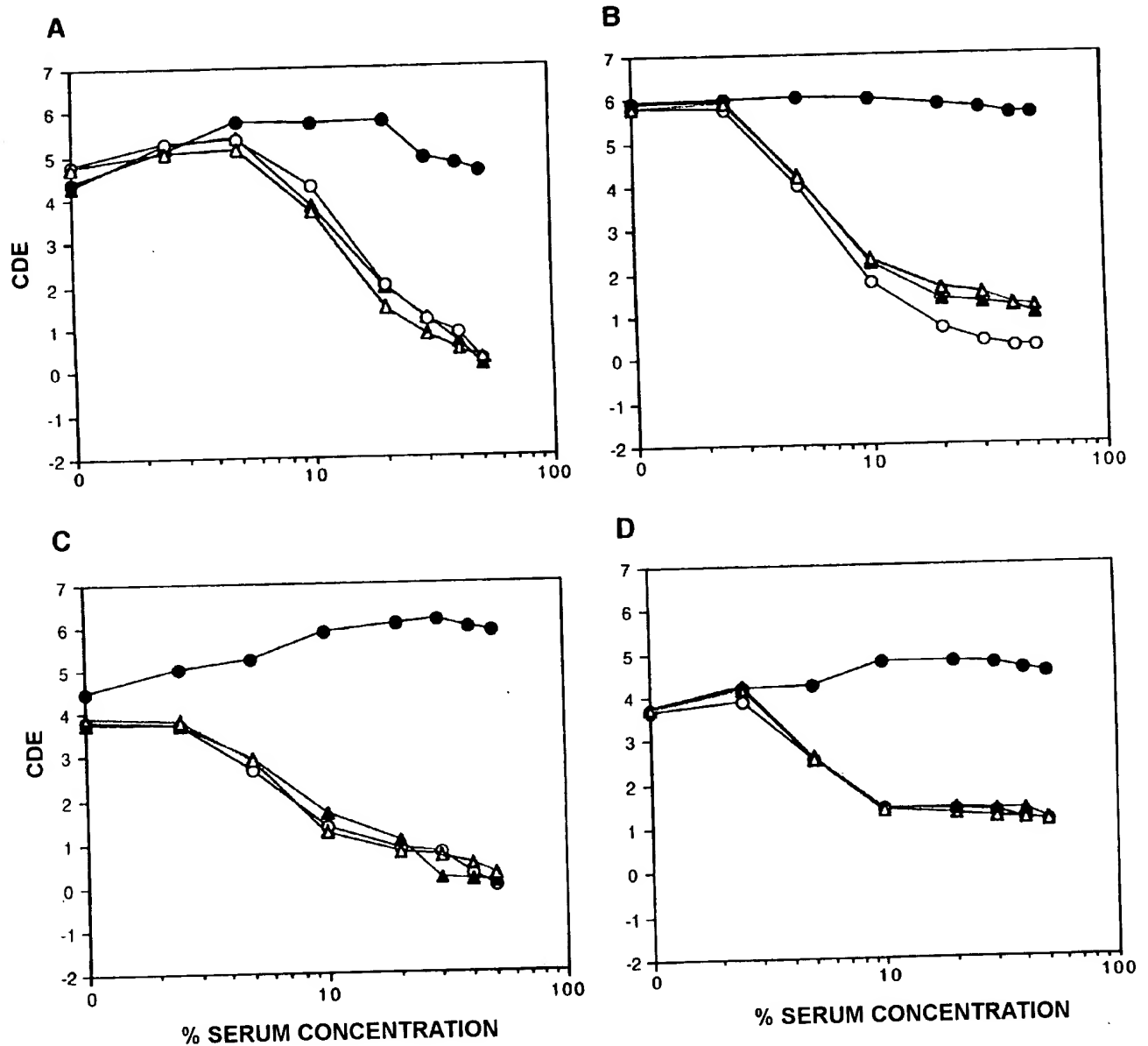
The cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each line tested was grown in serum alone (open circles) and in serum plus 50 ng/ml EGF (open triangles), 50 ng/ml TGF-alpha (closed triangles), or 10 nM E_2 without exogenous growth factors (closed circles). (A) - (D) show the results with the MCF-7A, MCF-7K, T47D, and ZR-75-1 cell lines, respectively.

FIGURE 28

EFFECT OF IGF-I ON THE GROWTH
OF HUMAN BREAST CANCER CELLS
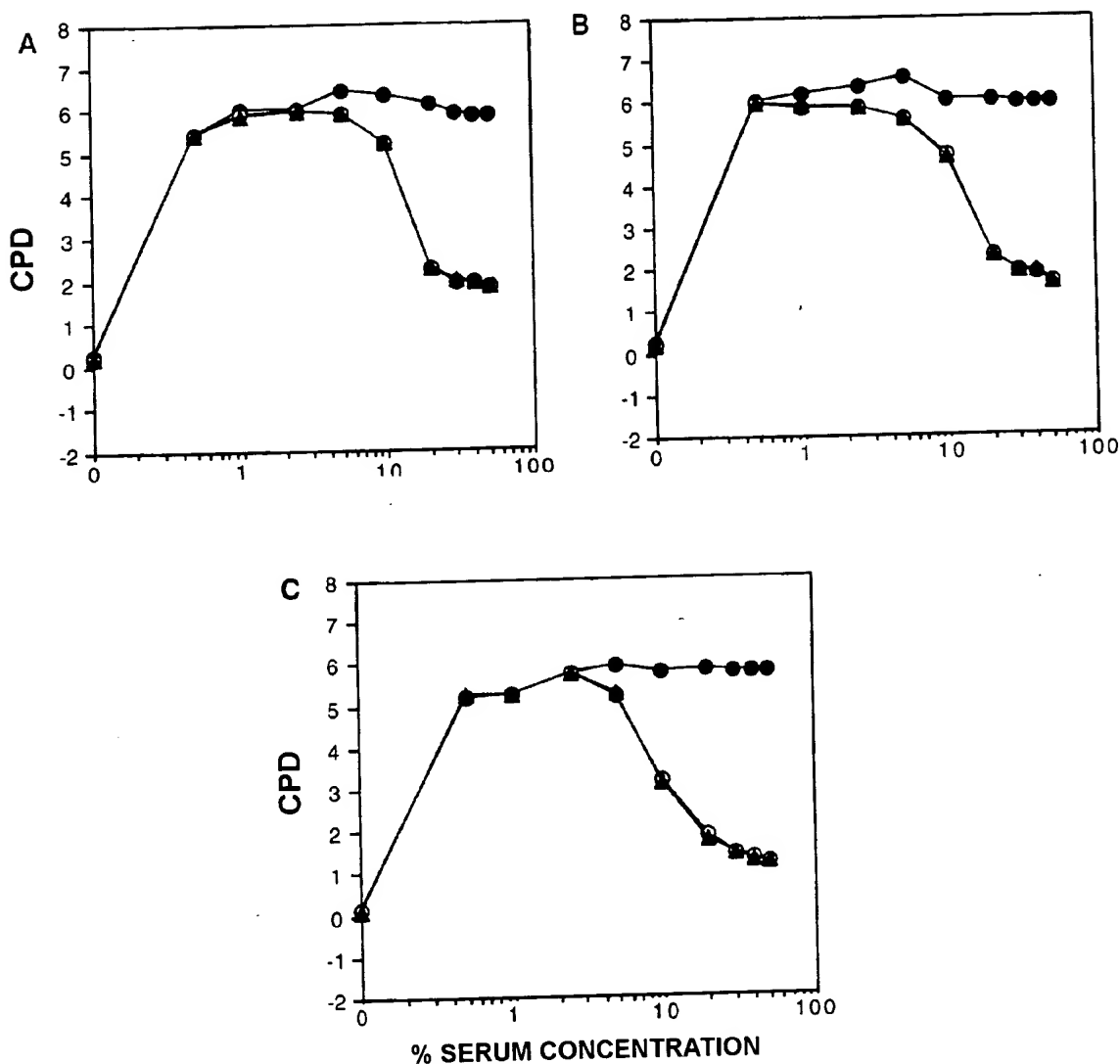
Breast cancer cells were grown in D-MEM/F-12 supplemented
with increasing concentrations of CDE horse serum. Each
cell line tested was grown in serum alone (open circles) and
in serum plus 1.0 ug/ml IGF-I (triangles), or in serum with
10 nM E_2 without exogenous growth factors (closed circles).
(A) - (C) show the results with the MCF-7K, MCF-7A and T47D
cells, respectively. Assays were conducted for 12-14 d.

FIGURE 29

T47D CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW FE" SERUM-FREE SERUM

LEGEND:

—■— "STANDARD" MEDIUM

—▲— "LOW-FE" MEDIUM

FIGURE 30

LNCaP CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW-FE" SERUM-FREE MEDIUM

DAY
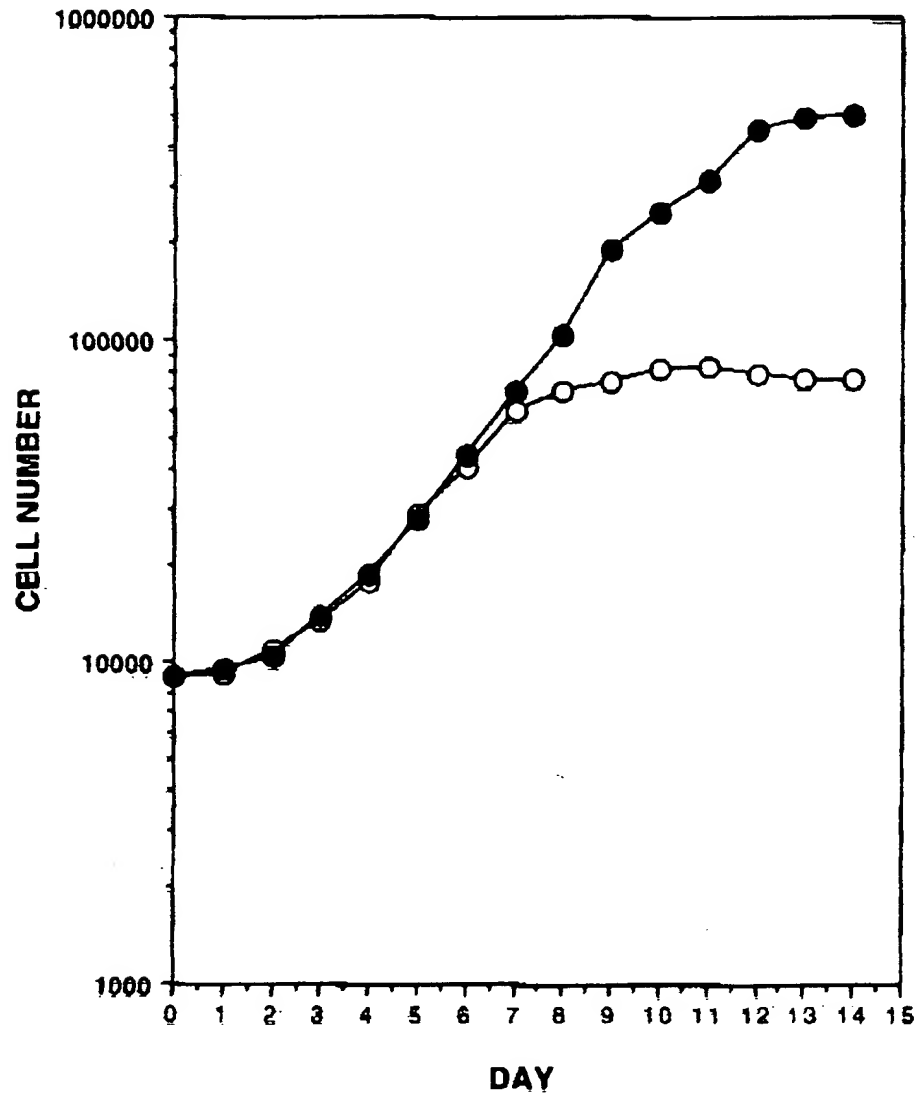
LEGEND:

—○— "STANDARD" MEDIUM

—●— "LOW-FE" MEDIUM

FIGURE 31

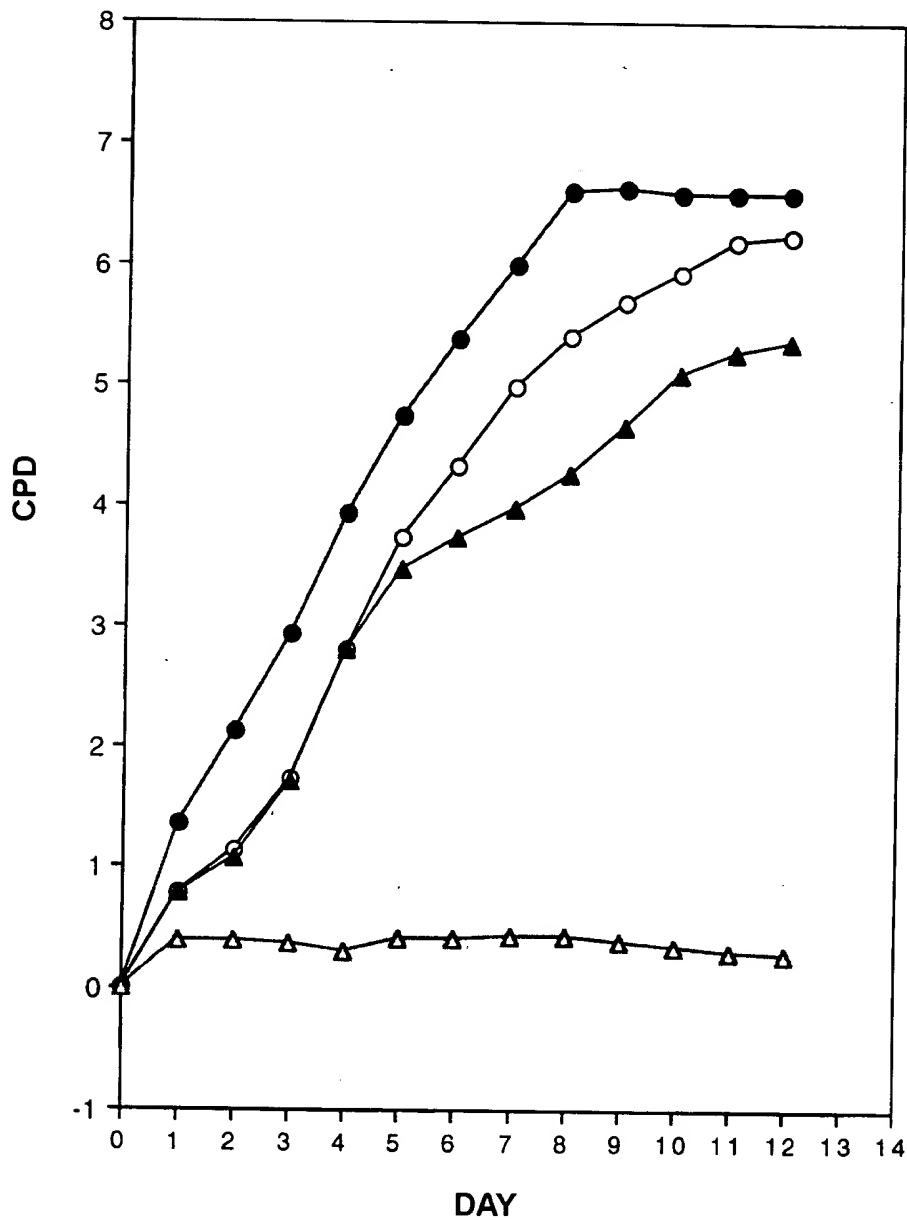## MDCK CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW FE" SERUM-FREE MEDIUM
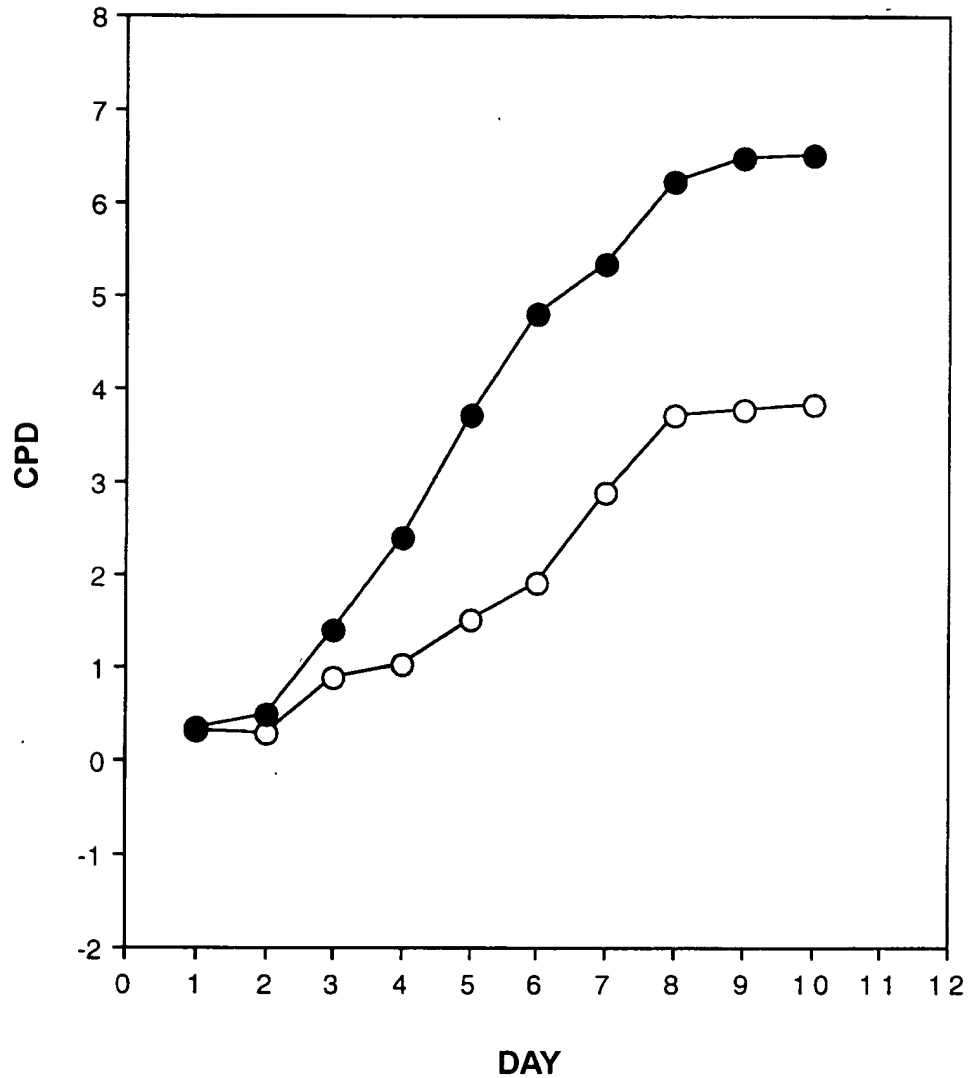
LEGEND:

—O— "STANDARD" MEDIUM

—●— "LOW-FE" MEDIUM

FIGURE 32

LNCaP CELL GROWTH IN CAPM ± DHT
AND 10% FETAL BOVINE SERUM

LEGEND:

Closed circles = Fetal bovine serum
Open circles = CAPM + DHT
Closed triangles = CAPM - DHT
Open triangles = D-MEM/F12 only

FIGURE 33

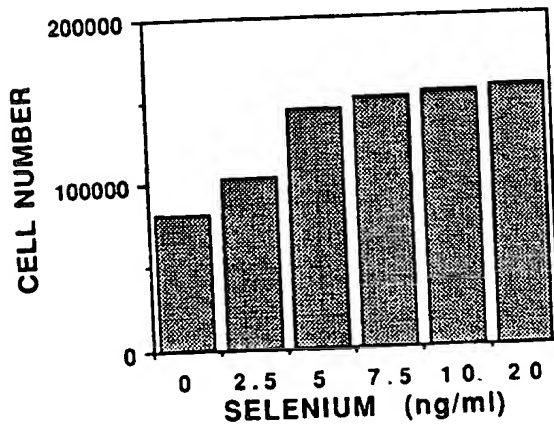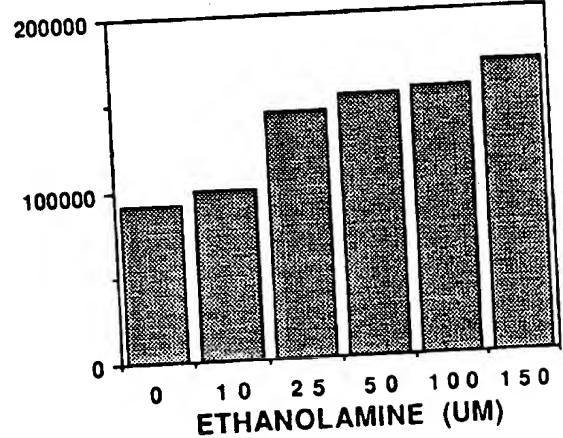## PC3 AND DU145 GROWTH IN SERUM - FREE MEDIUM VS MEDIUM WITH 10% FETAL CALF SERUM
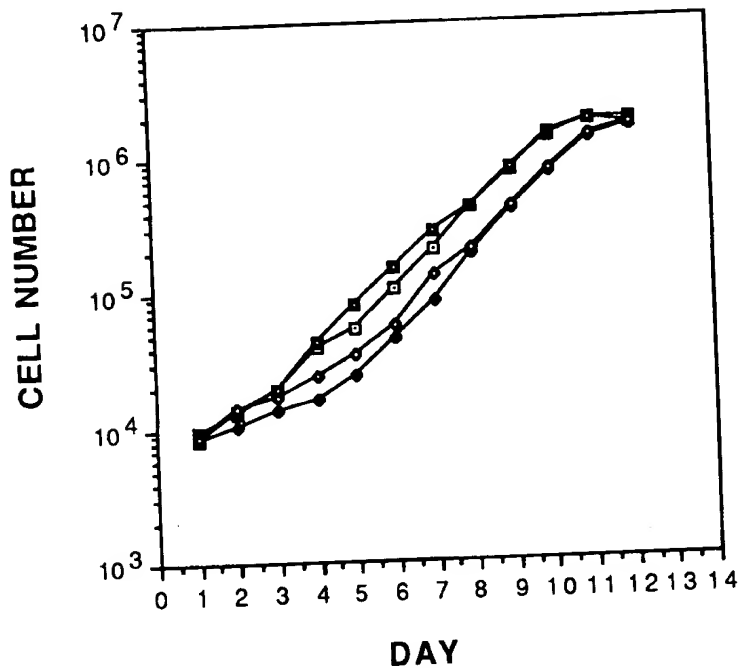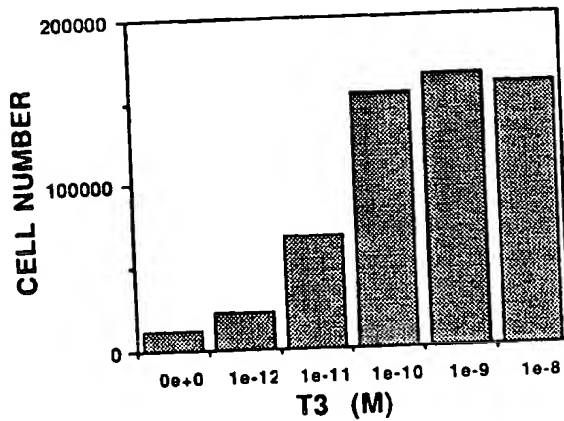

LEGEND:

_____▫_____ PC3 IN SERUM-FREE MEDIUM

_____♦_____ DU145 IN SERUM-FREE MEDIUM

_____■_____ PC3 IN 10% FETAL CALF SERUM
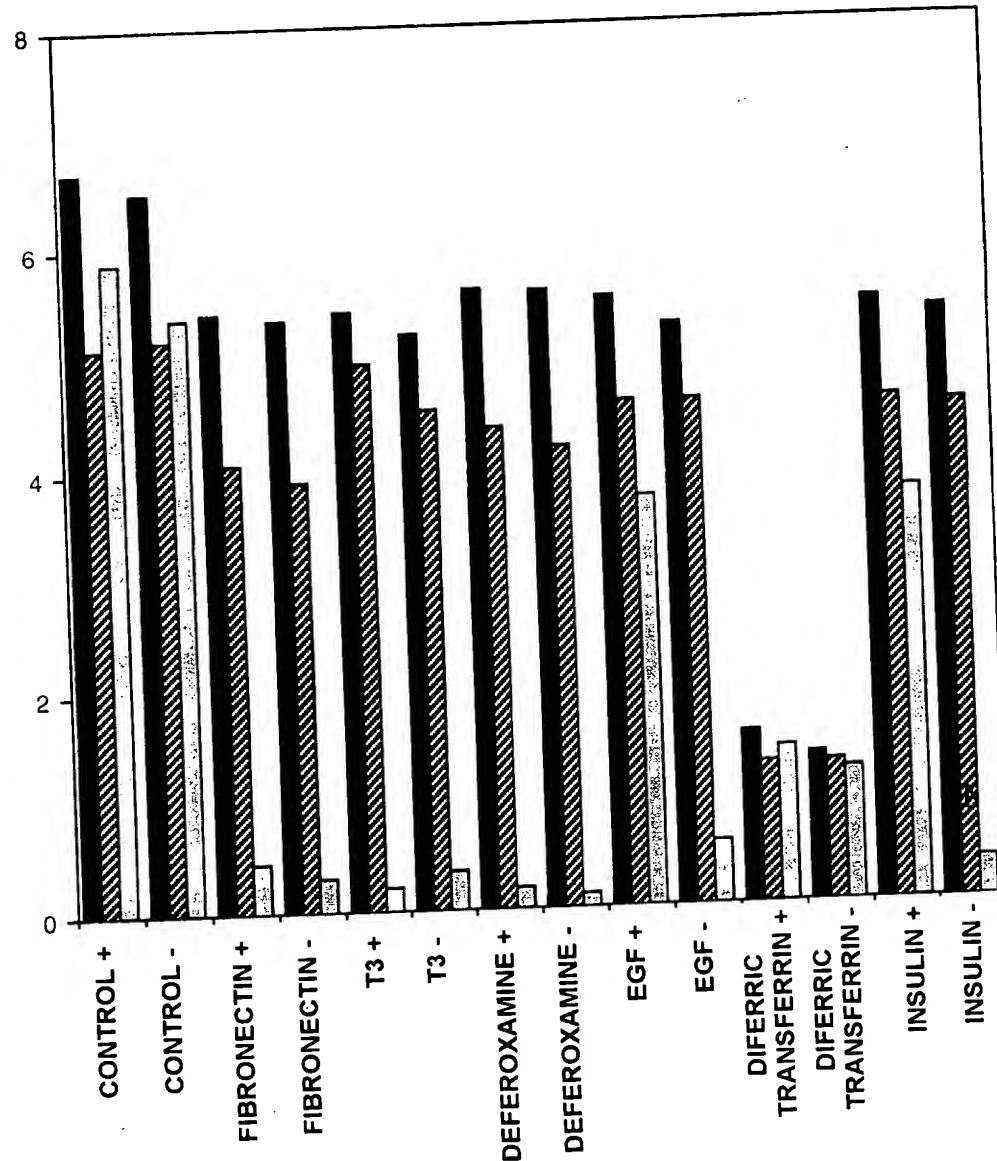
_____◇_____ DU145 IN 10% FETAL CALF SERUM

FIGURE 34

DOSE-RESPONSE EFFECTS OF INDIVIDUAL COMPONENTS OF CAPM SERUM-FREE MEDIUM ON LNCAP CELL GROWTH
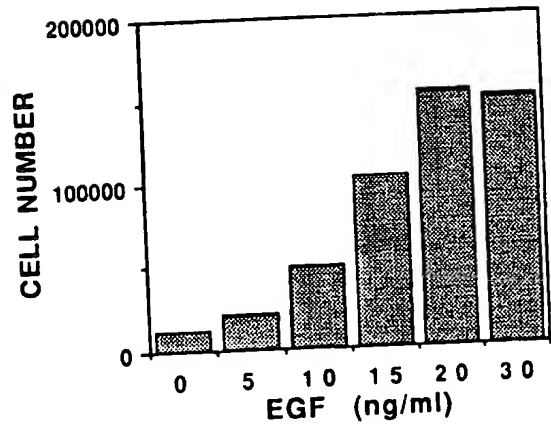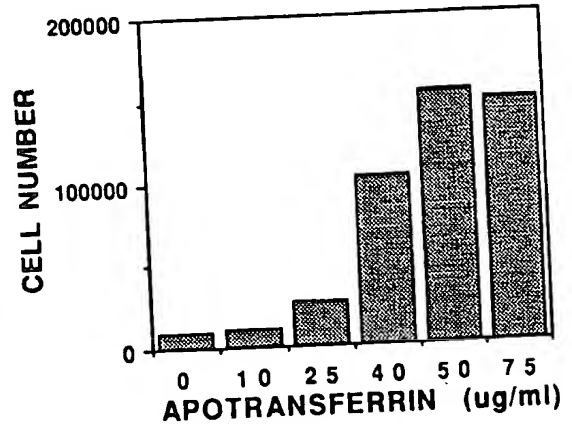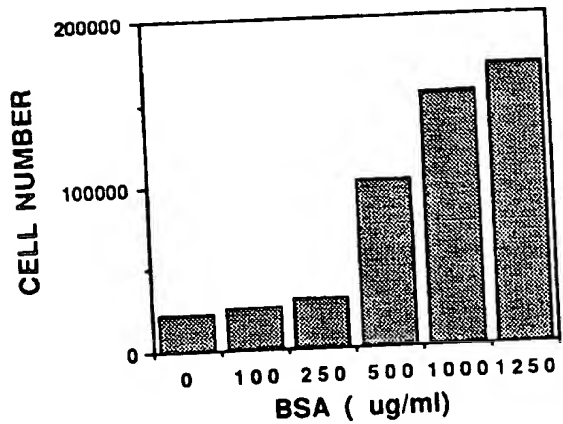
FIGURE 35

DELETIONS OF INDIVIDUAL COMPONENTS
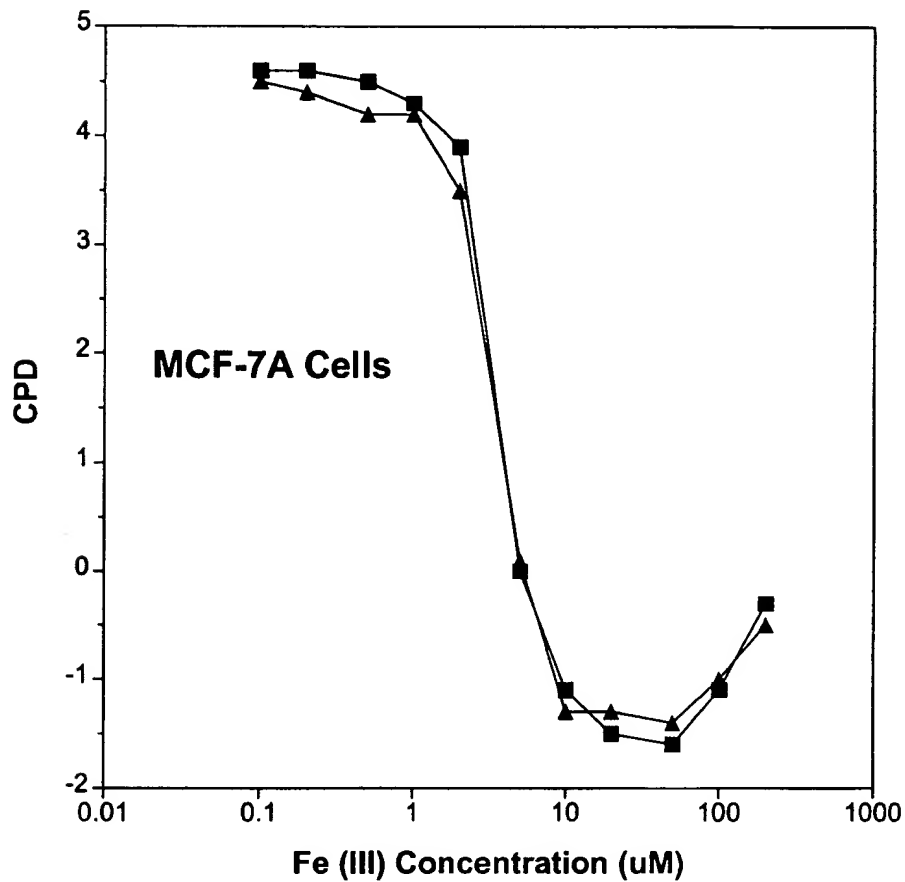### OF CAPM WITH PROSTATE CANCER CELL LINES

LEGEND: ■ = PC3

▨ = DU145

▢ = LNCaP

+ = 10 nM DHT

— = NO DHT

FIGURE 36

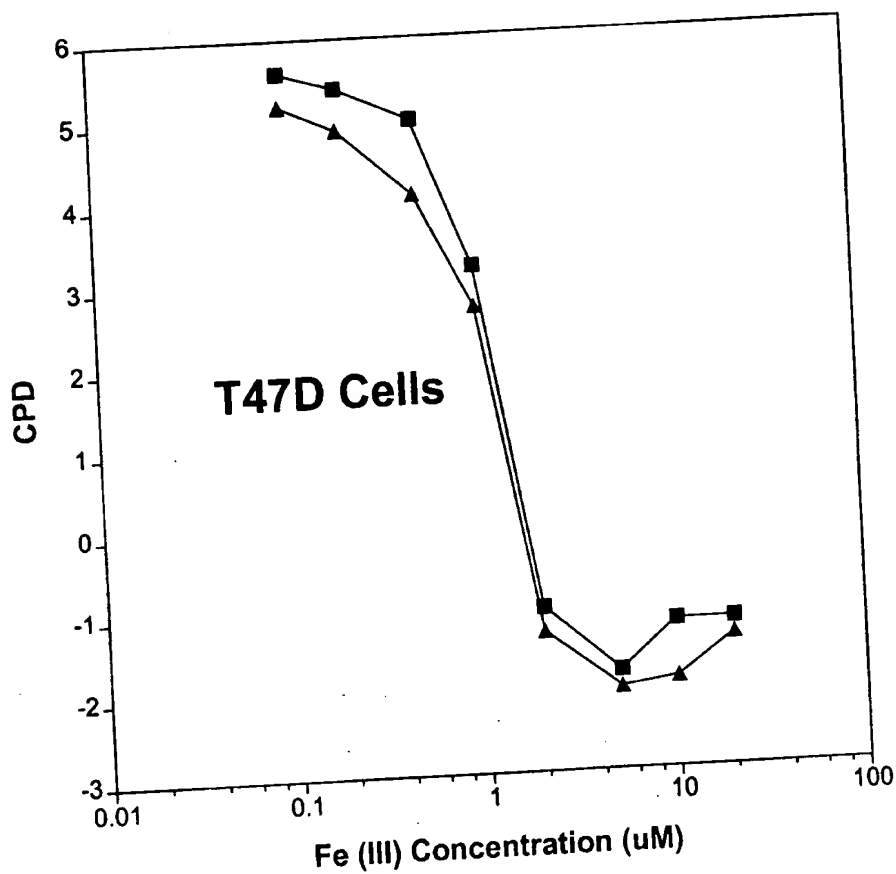## EFFECT OF FE (III) IN MCF-7A CELL GROWTH IN DDM-2MF DEFINED MEDIUM

MCF-7A Cells

LEGEND:

—■—   plus E_2

—▲—   minus E_2

FIGURE 37

EFFECT OF FE (III) IN T47D CELL GROWTH
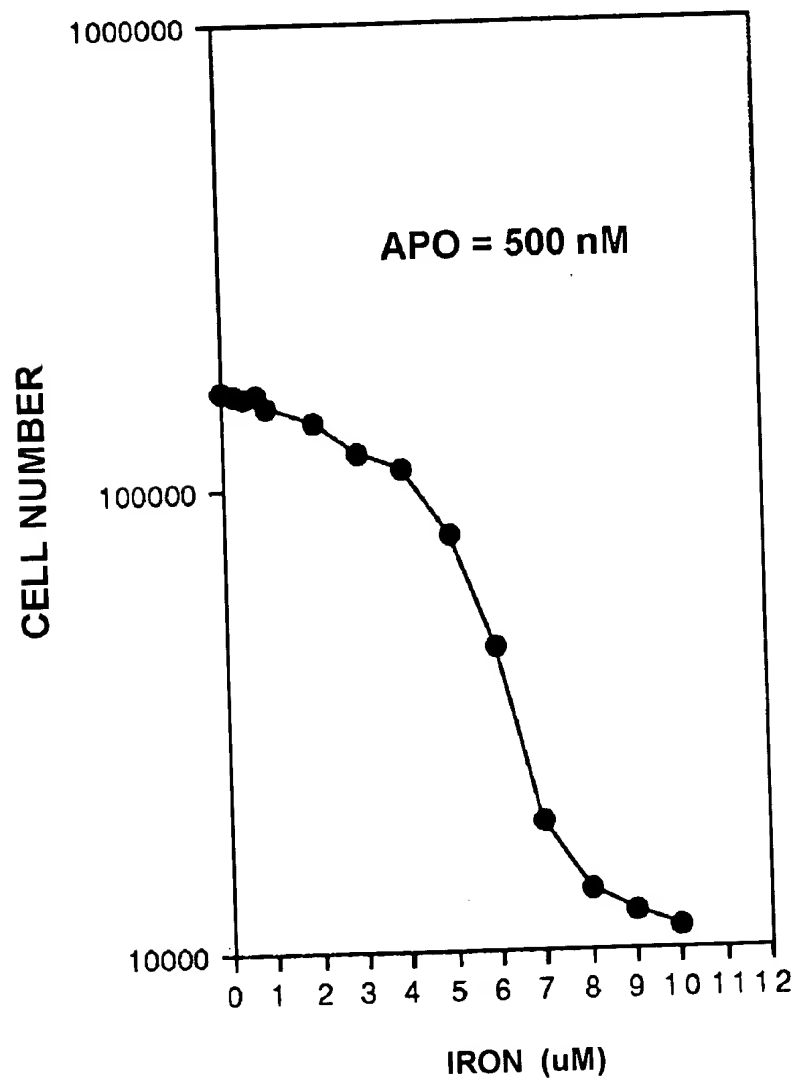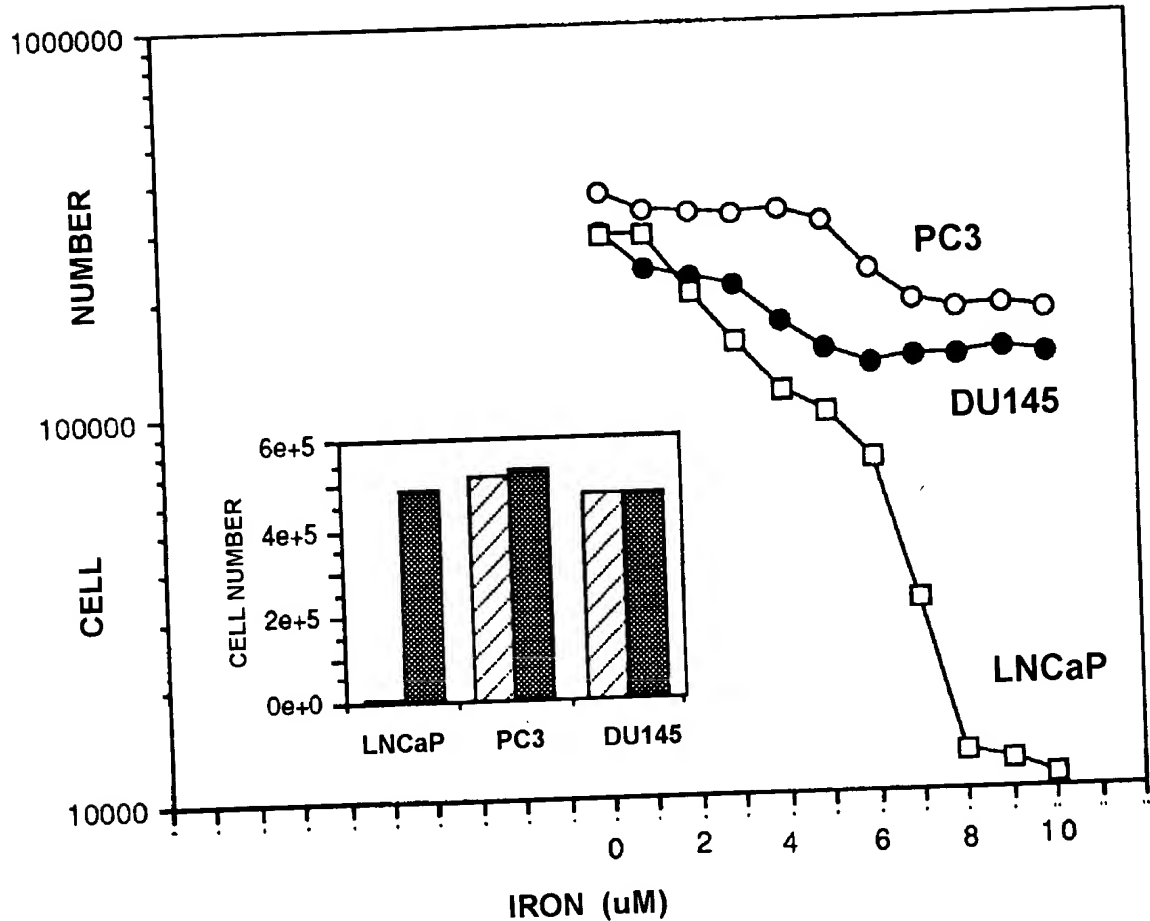## IN DDM-2MF DEFINED MEDIUM

LEGEND:

—■— plus E_2

—▲— minus E_2

FIGURE 38

EFFECTS OF INCREASING CONCENTRATIONS OF IRON ON LNCaP CELLS GROWN IN SERUM-FREE MEDIUM WITH APOTRANSFERRIN

APO = 500 nM

CELL NUMBER

IRON (uM)

FIGURE 39

EFFECTS OF IRON AND T_3 ON THREE PROSTATIC CELL LINES IN SERUM-FREE MEDIUM

INSERT:
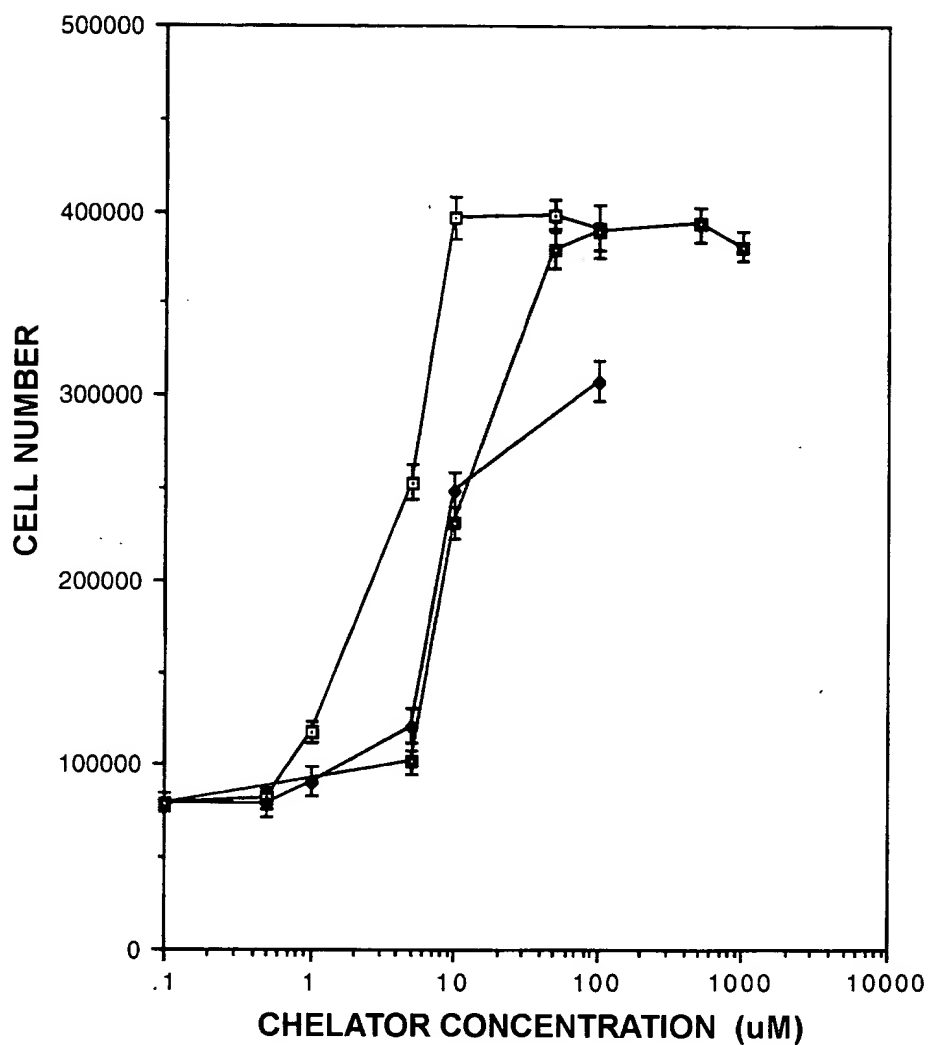
DARK BARS = GROWTH IN CAPM PLUS T_3

LIGHT (HATCHED) BARS = GROWTH IN CAPM MINUS T_3

NOTE THE STRIKING DEPENDENCE OF LNCaP CELLS ON T_3

FIGURE 40

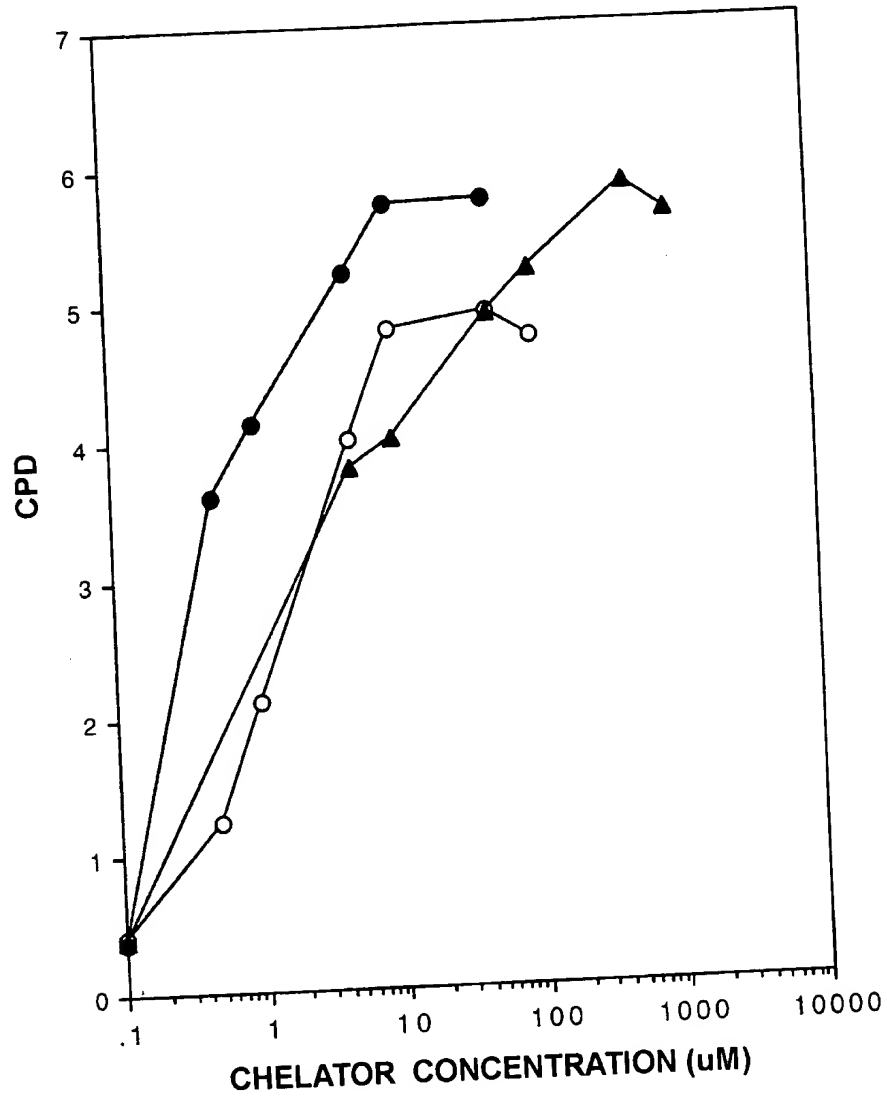## EFFECT OF CHELATORS ON SERUM-FREE T47D GROWTH UNDER HIGH IRON CONDITIONS

LEGEND:

——□—— DEFEROXAMINE

——◆—— EDTA

——▦—— CITRATE

FIGURE 41

EFFECT OF CHELATORS ON SERUM-FREE LNCaP GROWTH UNDER HIGH IRON CONDITIONS
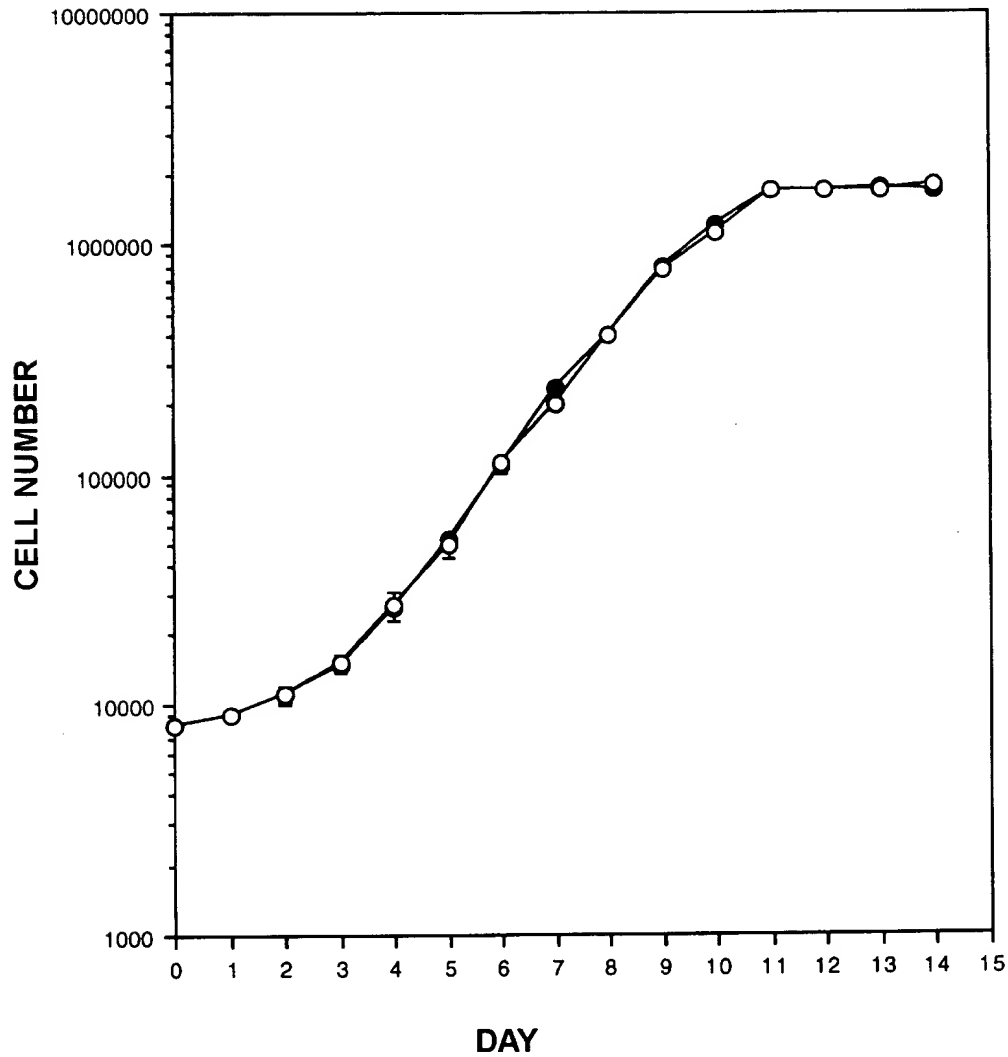

LEGEND:

Closed circles = Deferoxamine

Open circles = Citrate

Closed triangles = EDTA

FIGURE 42

DU145 GROWTH IN SERUM-FREE MEDIUM
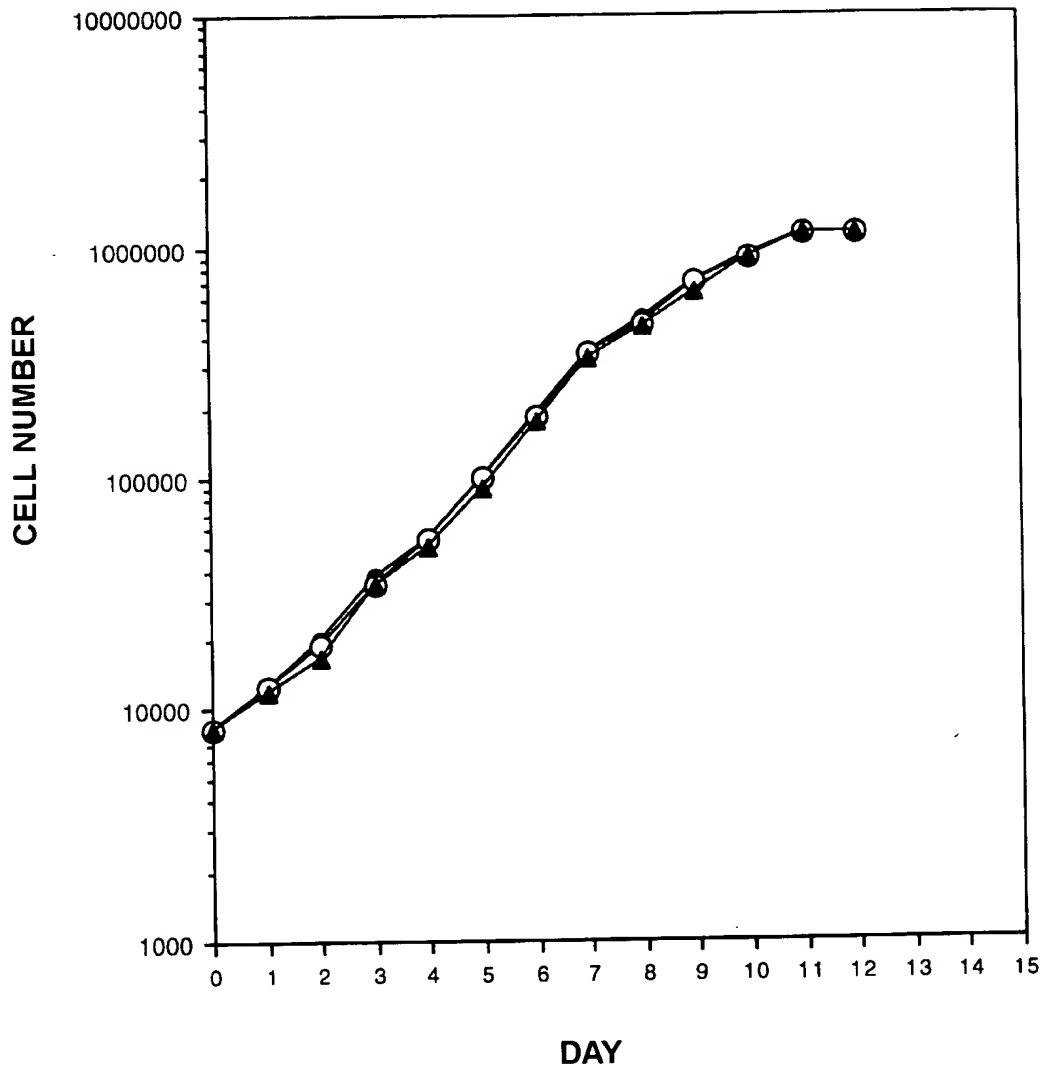## BASED ON "LOW FE" OR "STANDARD" MEDIUM

LEGEND:

Open circles = "Low Fe" medium

Closed circles = "Standard" medium

FIGURE 43

PC3 GROWTH IN SERUM-FREE MEDIUM
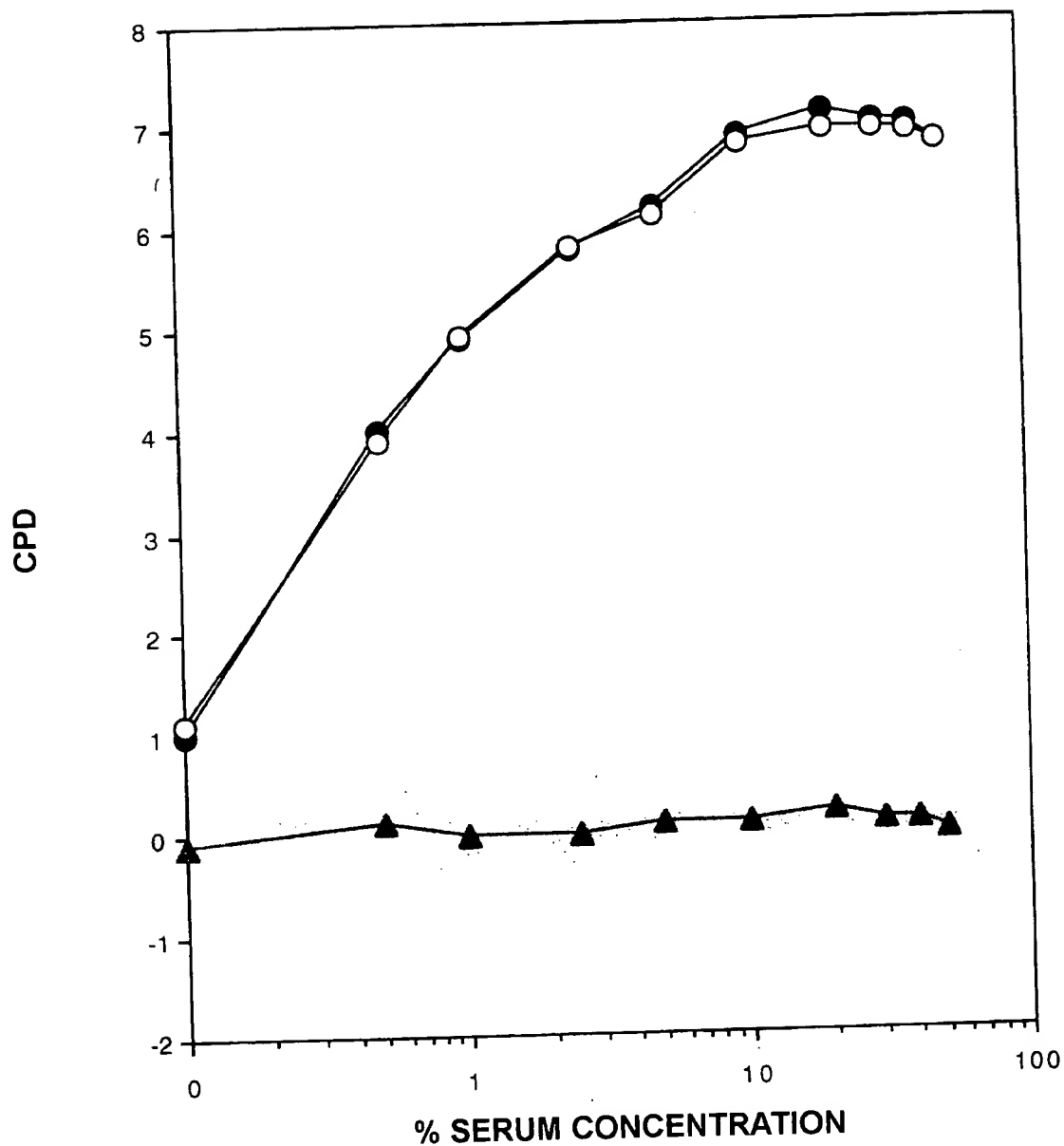## BASED ON "LOW FE" OR "STANDARD" MEDIUM

LEGEND:

Open circles = "Low Fe" medium

Closed triangles = "Standard" medium
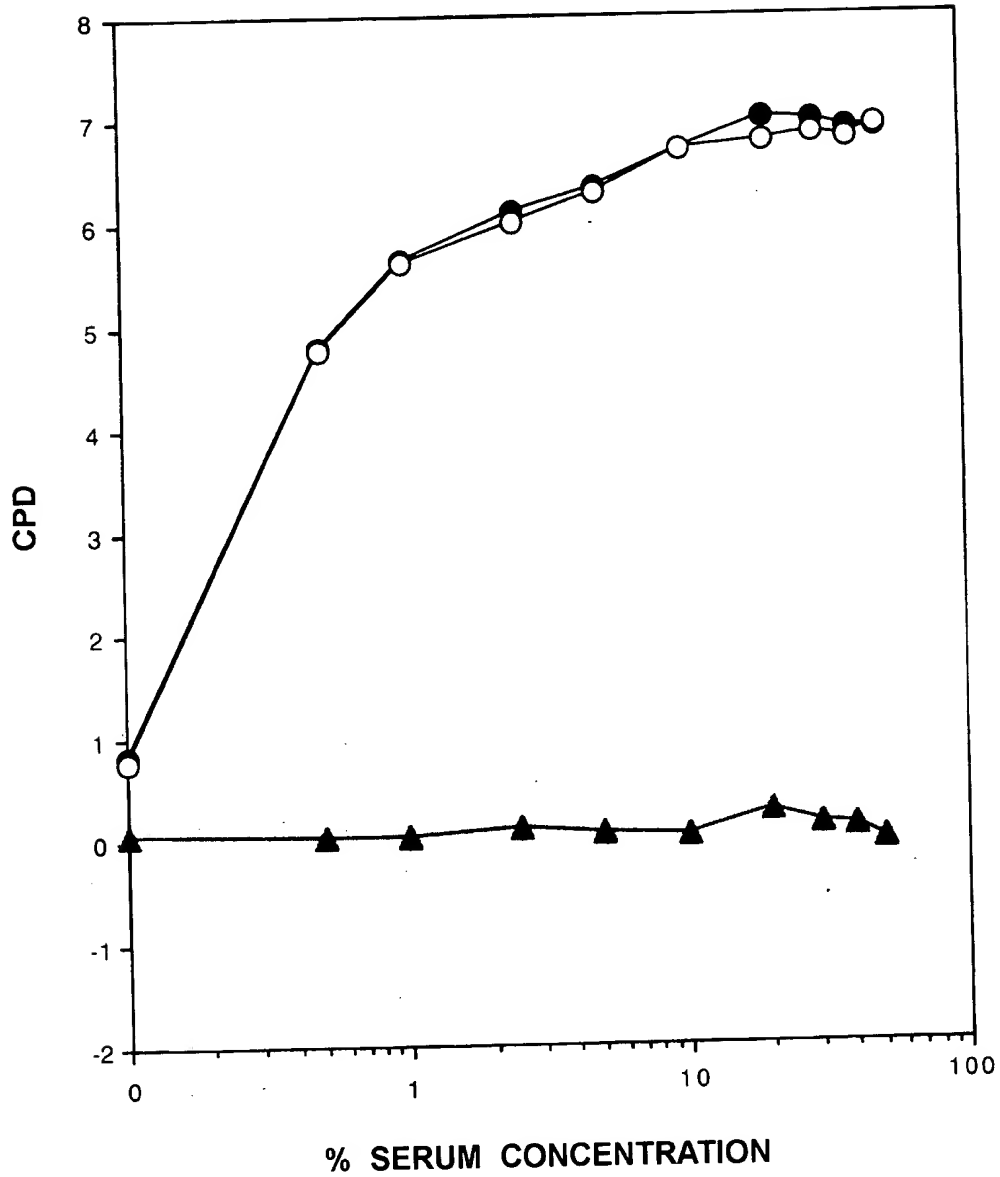
FIGURE 44

CDE HORSE SERUM TITRATION ON DU145 CELLS

LEGEND:

—●— = + 10 nM DHT

—○— = STEROID FREE

—▲— = ANDROGENIC EFFECT
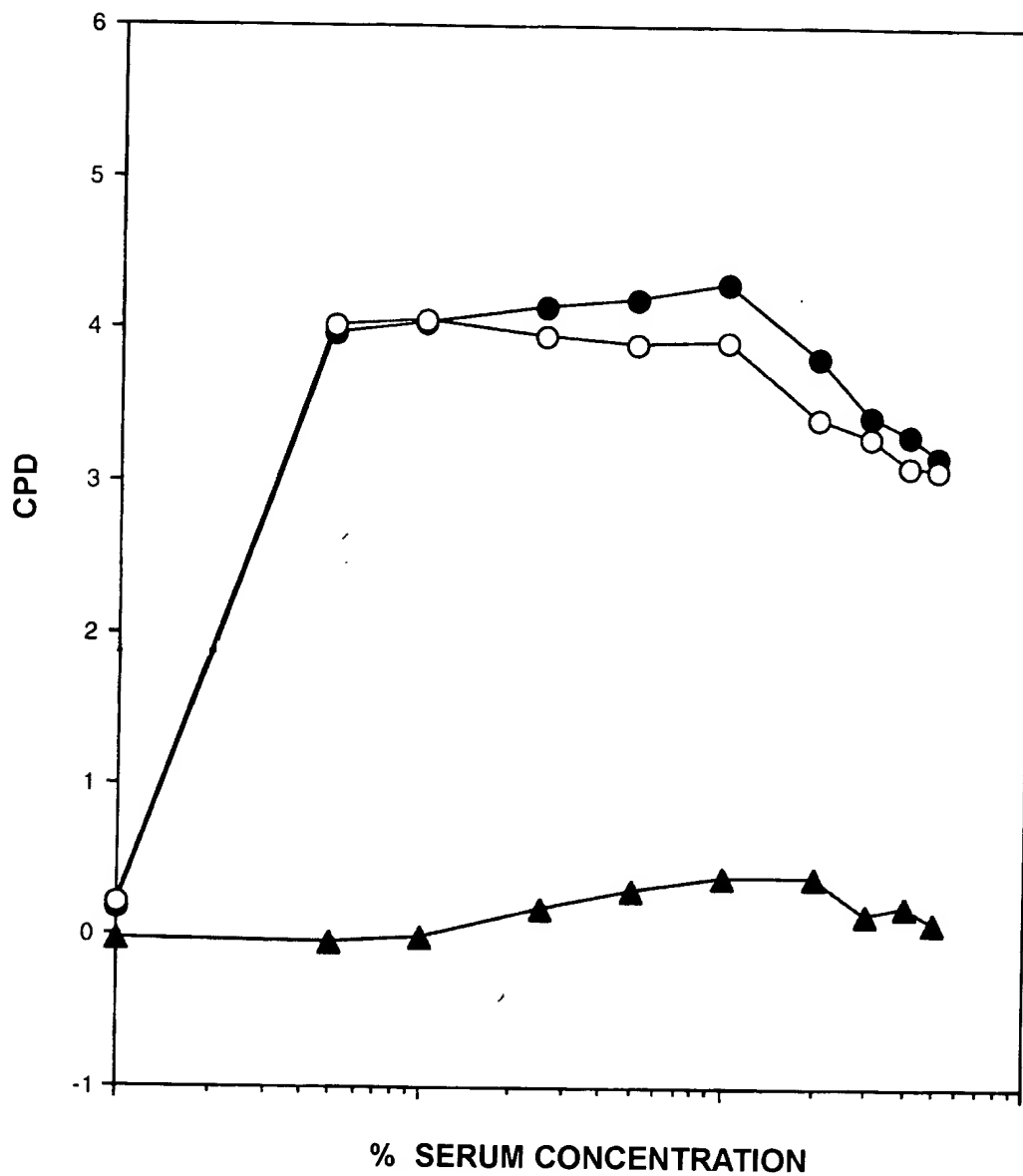
FIGURE 45

CDE HORSE SERUM TITRATION ON PC3 CELLS

LEGEND:

—●— = + 10 nM DHT

—○— = STEROID FREE

—▲— = ANDROGENIC EFFECT

FIGURE 46

CDE HORSE SERUM TITRATION ON ALVA-41 CELLS
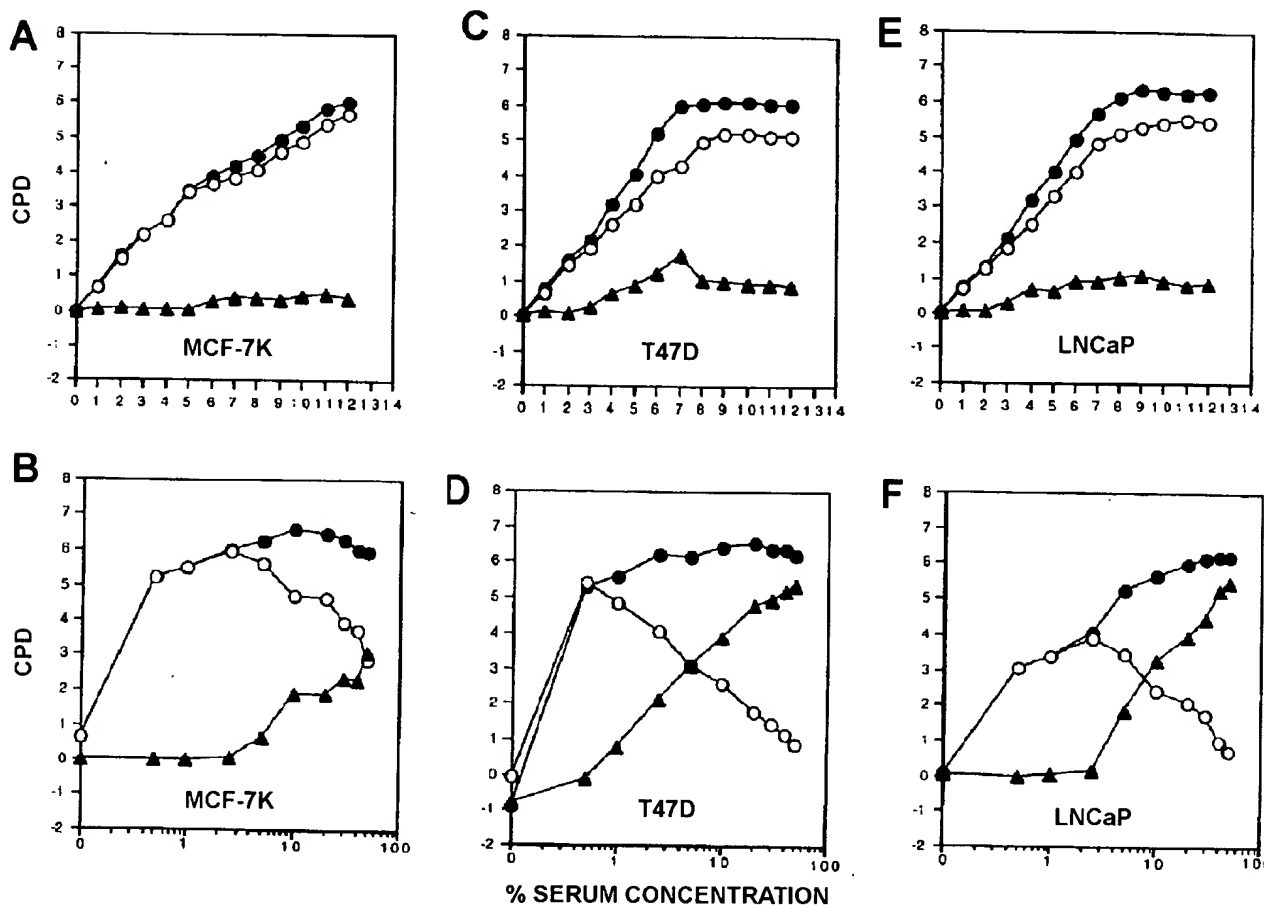

LEGEND:

●— = + 10 nM DHT

○— = STEROID FREE

▲— = ANDROGENIC EFFECT

FIGURE 47

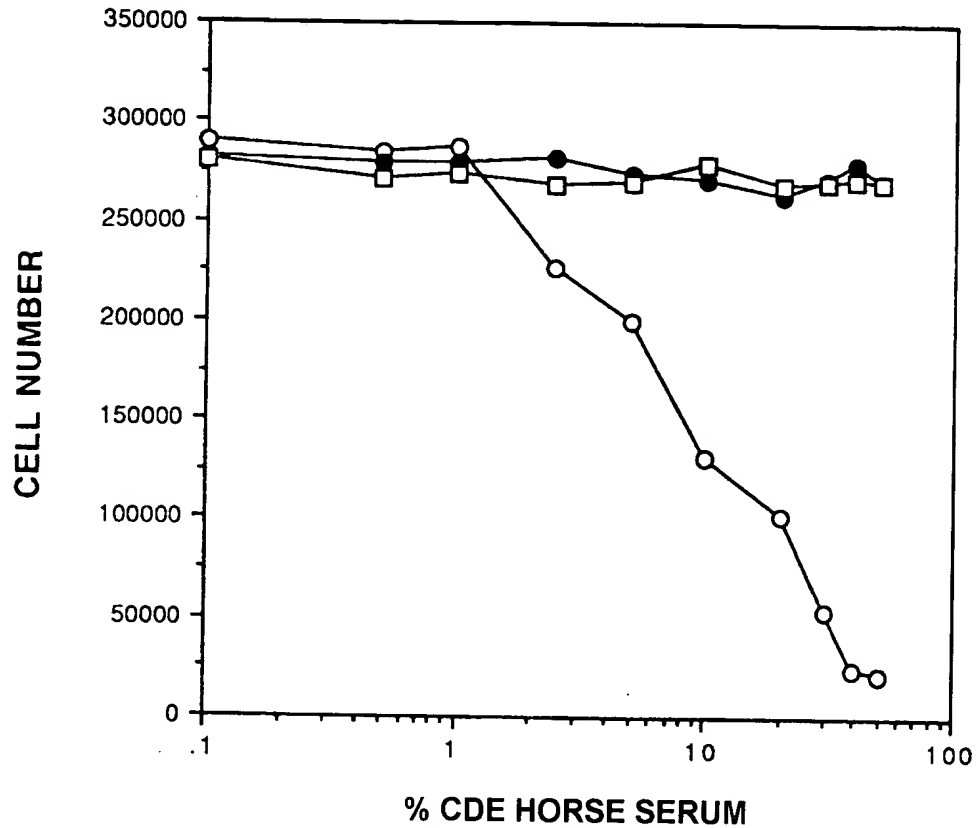## EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE HUMAN TUMOR CELL GROWTH

The cells were grown in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) MCF-7K cell growth was measured daily in serum-free defined DDM-2MF with 10 nM E_2 (closed circles) and without steroid (open circles) E_2. Triangles = estrogenic effect.

(B) MCF-7K cell growth measured after 12 d in D-MEM-F-12 supplemented with the designated concentrations of serum with E_2 (closed circles) and without steroid (open circles). The estrogenic effect is shown by triangles.

(C) and (D) show the same experiments as in (A) and (B), respectively, except with T47D cells.

(E) and (F) show the same experiments as in (A) and (B), respectively, xcept with LNCaP cells. In (E) the serum-free medium was CAPM.

FIGURE 48

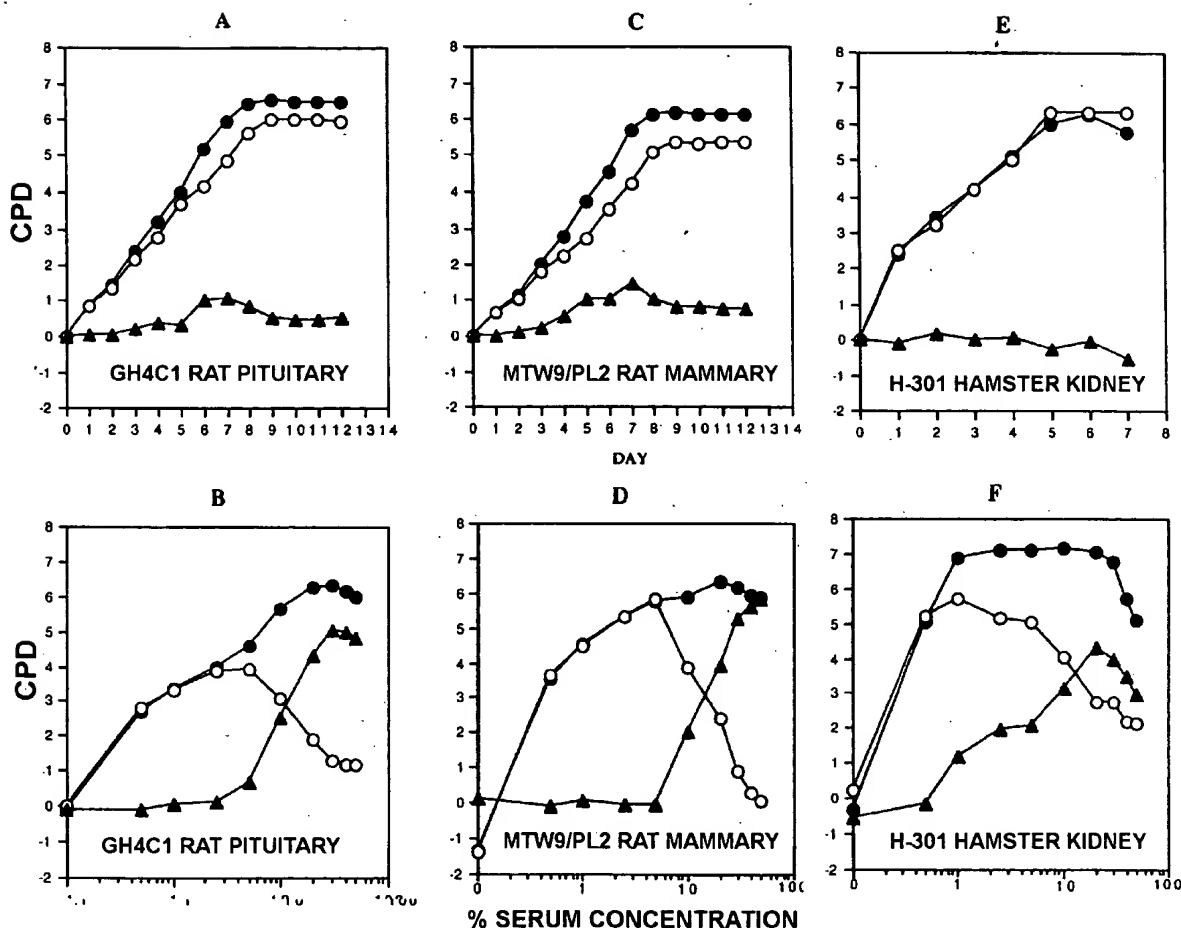## CDE HORSE SERUM TITRATION ON LNCaP
GROWTH IN SERUM FREE CONDITIONS

LEGEND:

—o— NO STEROID

—●— + E_2

—□— + DHT

FIGURE 49

## EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE RODENT TUMOR CELL GROWTH

Comparison of the effects of estrogen on steroid hormone-responsive rodent tumor cell growth in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) GH_4C_1 rat pituitary tumor cell growth measured daily in serum-free PCM-9 with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles.
(B) $GH_4 C_1$ cell growth measured after 9 d in D-MEM-F-12 supplemented with the designated concentrations of CDE horse serum with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles.
(C) and (D) show the same experiments as in (A) and (B) respectively, but with th MTW9/PL2 rat mammary tumor cells. The serum-free medium in (D) was DDM-2A.
(E) and (F) show the same xperiments as in (A) and (B), resp ctively, except with the H-301 hamster kidney tumor cells. In (E) the serum-free m dium was CAPM.

FIGURE 50

THE EFFECT OF DHT, E_2, AND DES ON LNCaP CELLS GROWN IN CDE HORSE SERUM


% SERUM CONCENTRATION
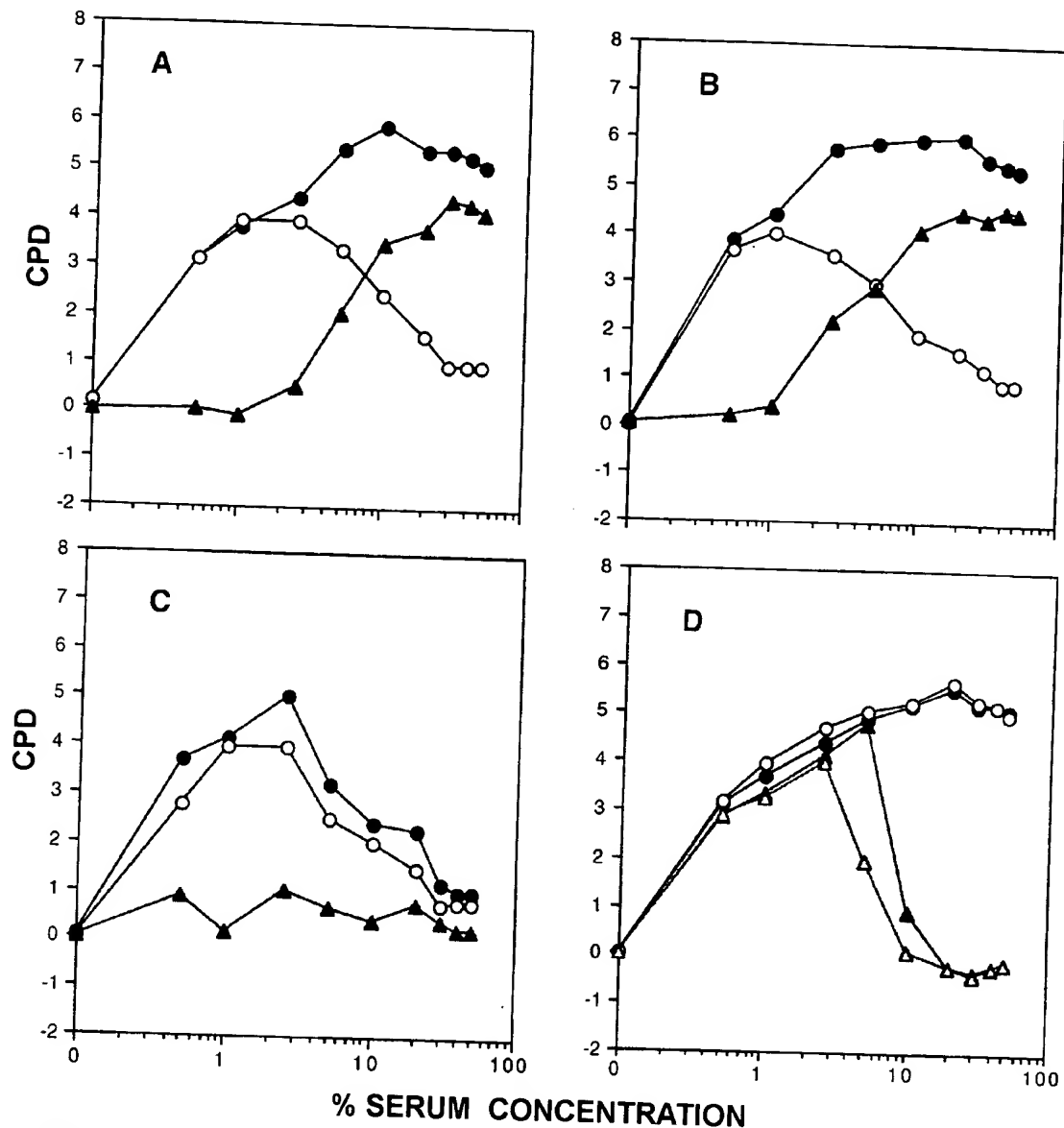
LEGEND:

(A) Open circles = - DHT
  Closed circles = + DHT
  Closed trianges = Androgenic effect

(B) Open circles = - E_2
  Closed circles = + E_2
  Closed triangles = Estrogenic eff ct

(C) Open circles = - DES
  Closed circles = + DES
  Closed trianles = Estrogenic effect

(D) Open circles = DHT & DES
  Closed circles = E_2 & DES
  Open triangles = No additions
  Closed triangles = DES only

FIGURE 51

PHENYL SEPHAROSE ELUTION OF

CBG (CA-PS-POOL 1) AND SHBG-LIKE (CA-PS-POOL 11)
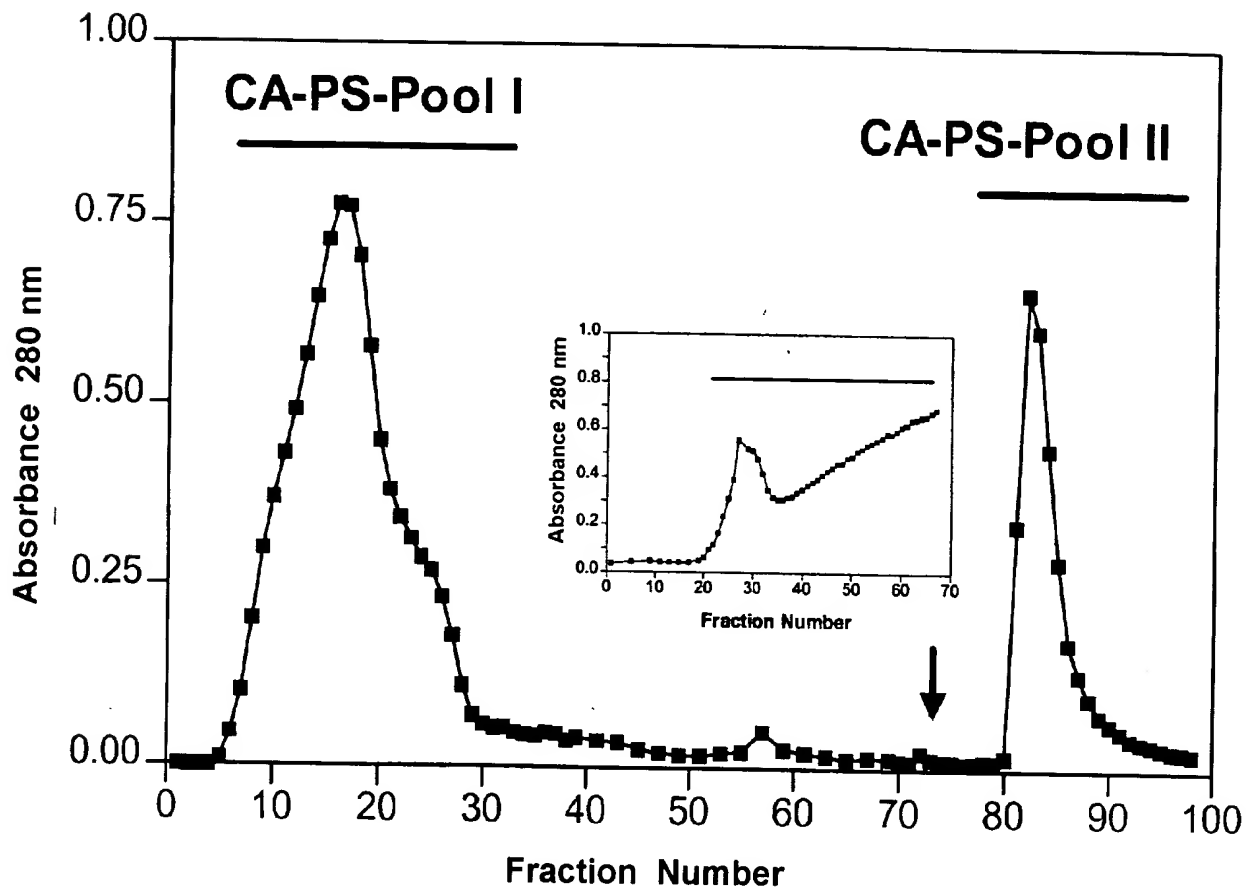
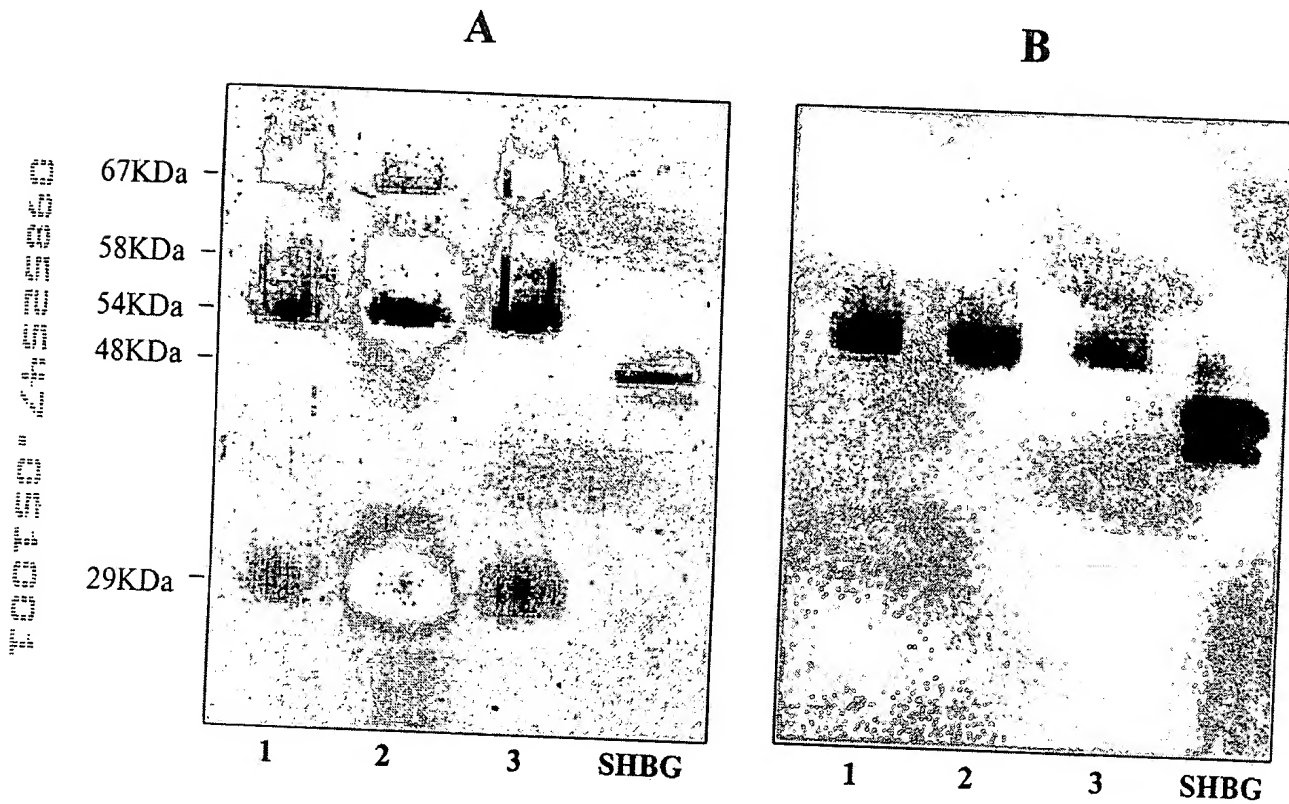**ARROW = ELUTION WITH 40% ETHYLENE GLYCOL**

INSERT: CORTISOL AFFINITY COLUMN ELUTION

BARS = POOLED ACTIVE FRACTION

FIGURE 52

SDS PAGE (A) AND WESTERN ANALYSIS (B) OF THREE
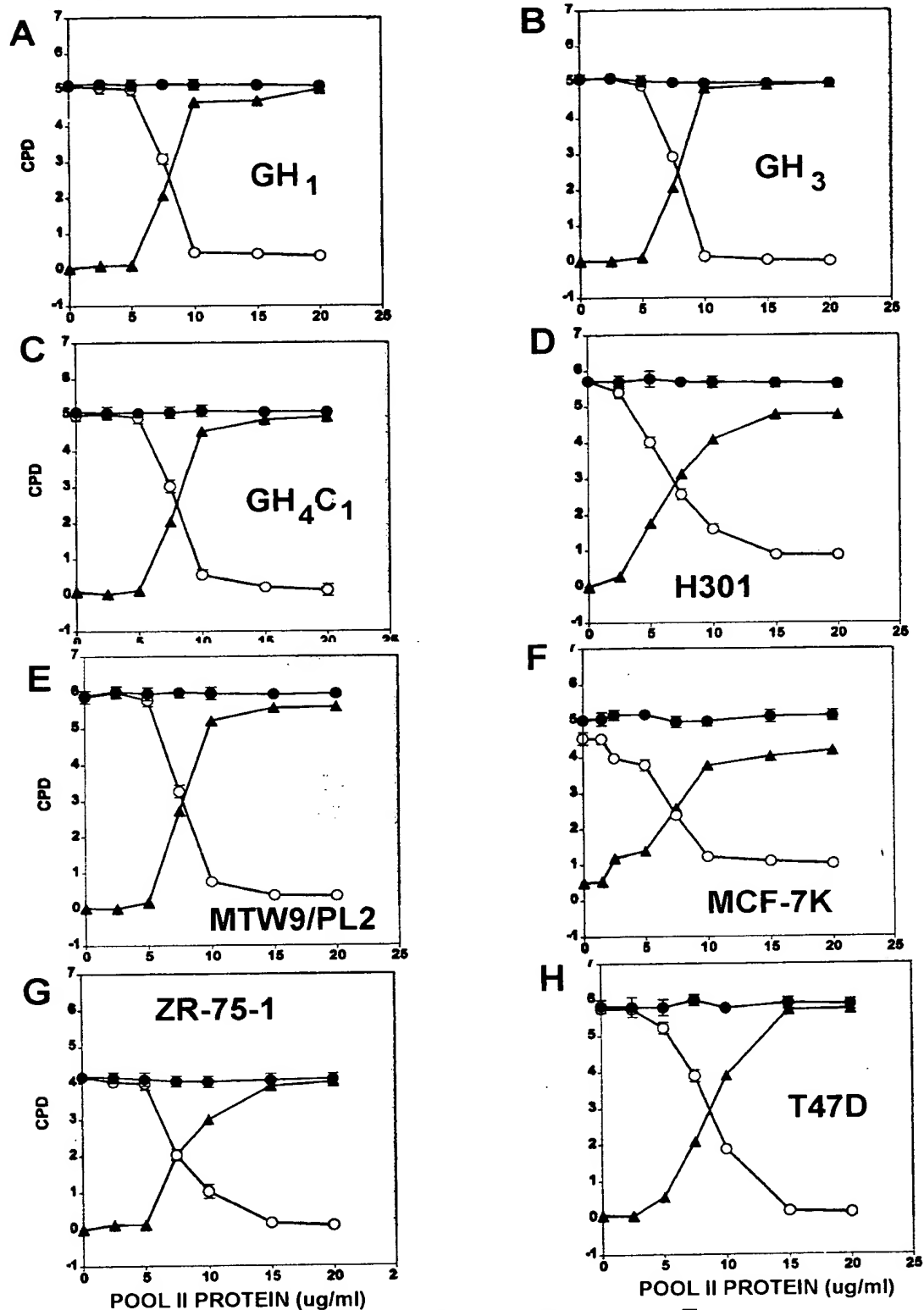## PREPARATIONS OF CA-PS-POOL II VS HUMAN SHBG

LANES 1, 2, AND 3 = 10 ug each of CA-PS-POOL II

LANE "SHBG" = 10 mg of purified protein

FIGURE 53

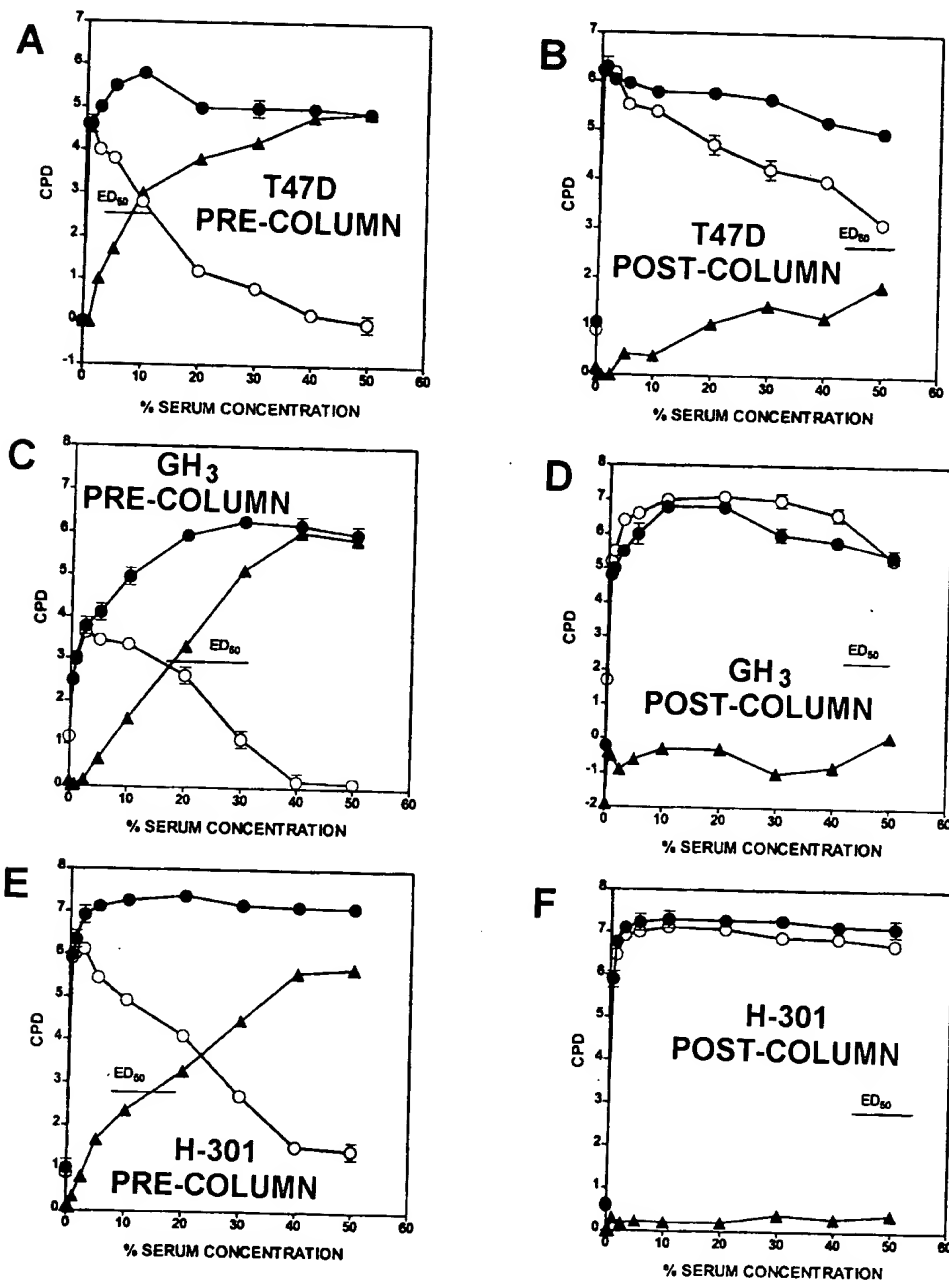## ASSAY OF CA-PS-POOL II ESTROGEN REVERSIBLE INHIBITORY ACTIVITY WITH SEVERAL ER⁺CELL LINES

LEGEND:

Open circles = − E_2

Clos d circles = + E_2

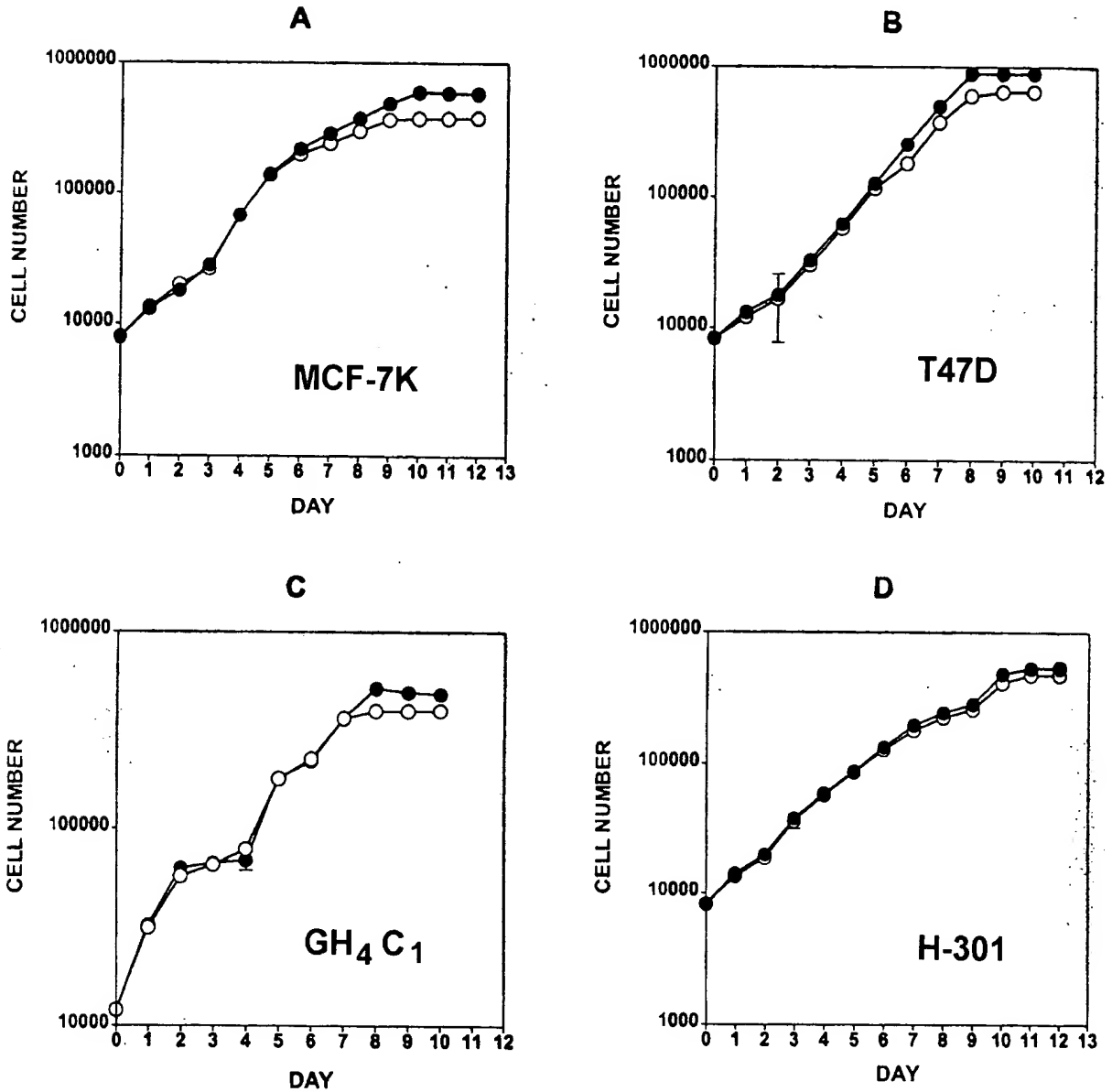Closed triangl s = Estrog nic effect

FIGURE 54

CORTISOL-AGAROSE AFFINITY REMOVAL OF THE INHIBITOR FROM CDE-SERUM

LEGEND: Open circles = – E_2
Closed circl s = + E_2
Closed triangles = Estrogenic effect

FIGURE 55

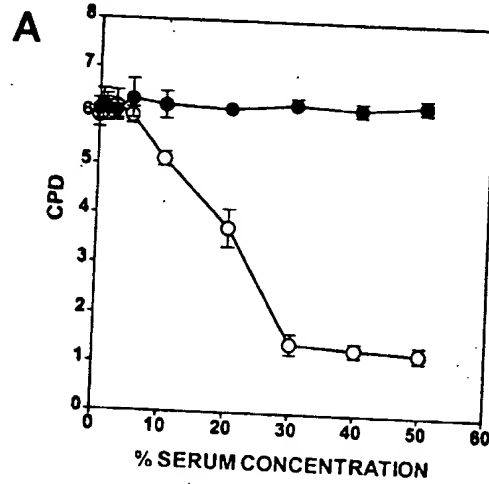## GROWTH OF ER$^+$ CELL LINES IN SERUM-FREE MEDIUM $\pm$ E$_2$
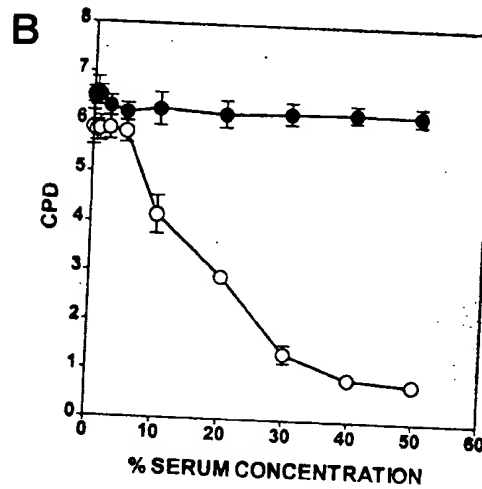
A

B

C

D

LEGEND:

Closed circles = + E$_2$

Open circles = – E$_2$

FIGURE 56

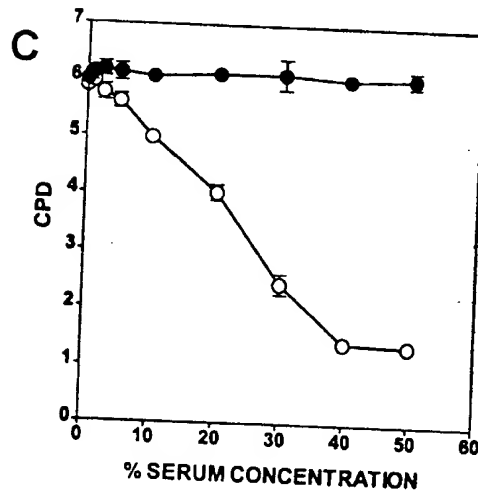## EFFECT OF CDE-SERUM ON ESTROGEN RESPONSIVE GROWTH OF THREE ER+ CANCER CELL LINES IN SFM

A =

T47D IN DDM-2MF

B =

MTW9/PL2 IN DDM-2A

C =

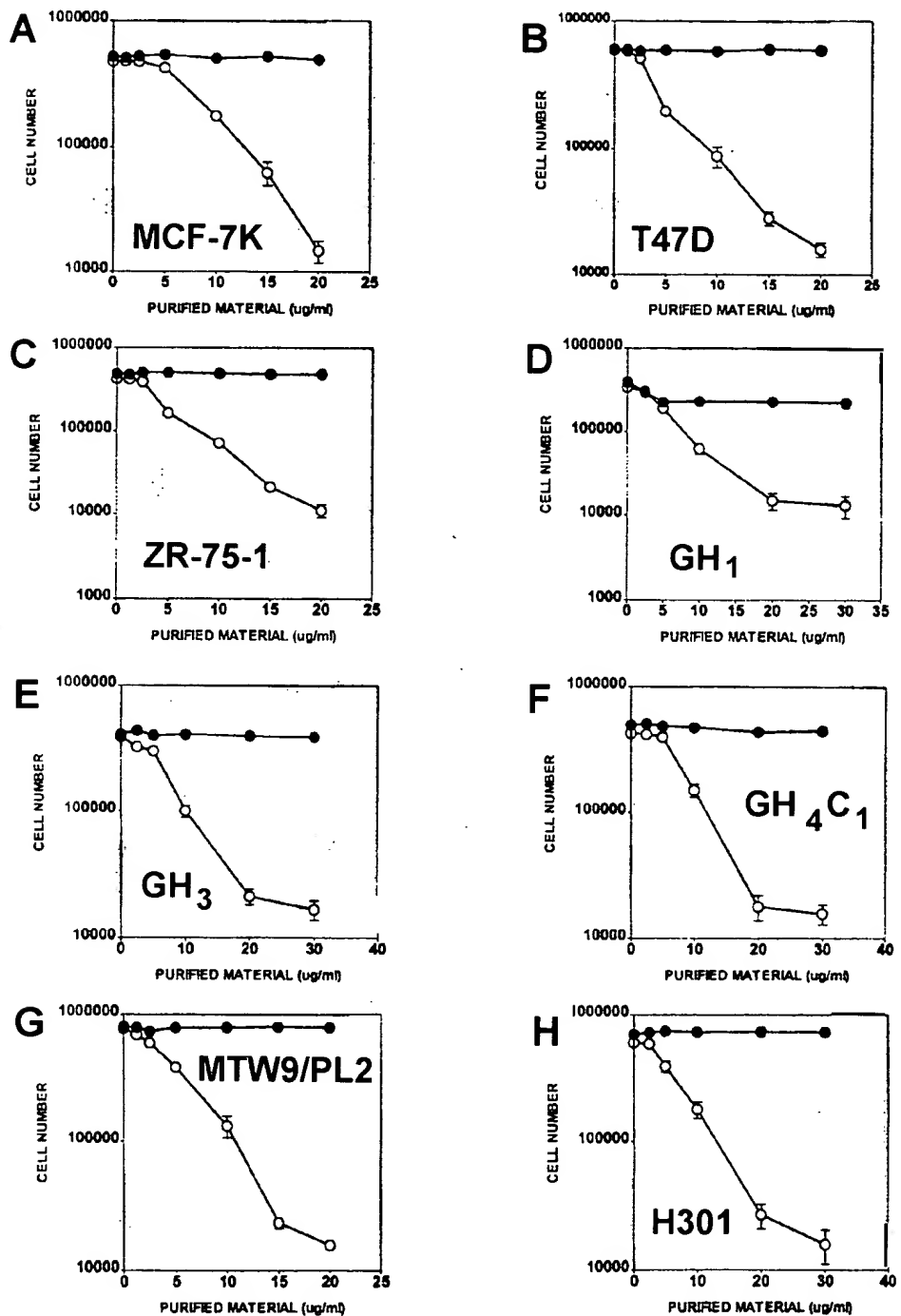$GH_4 C_1$ IN PCM 9

FIGURE 57

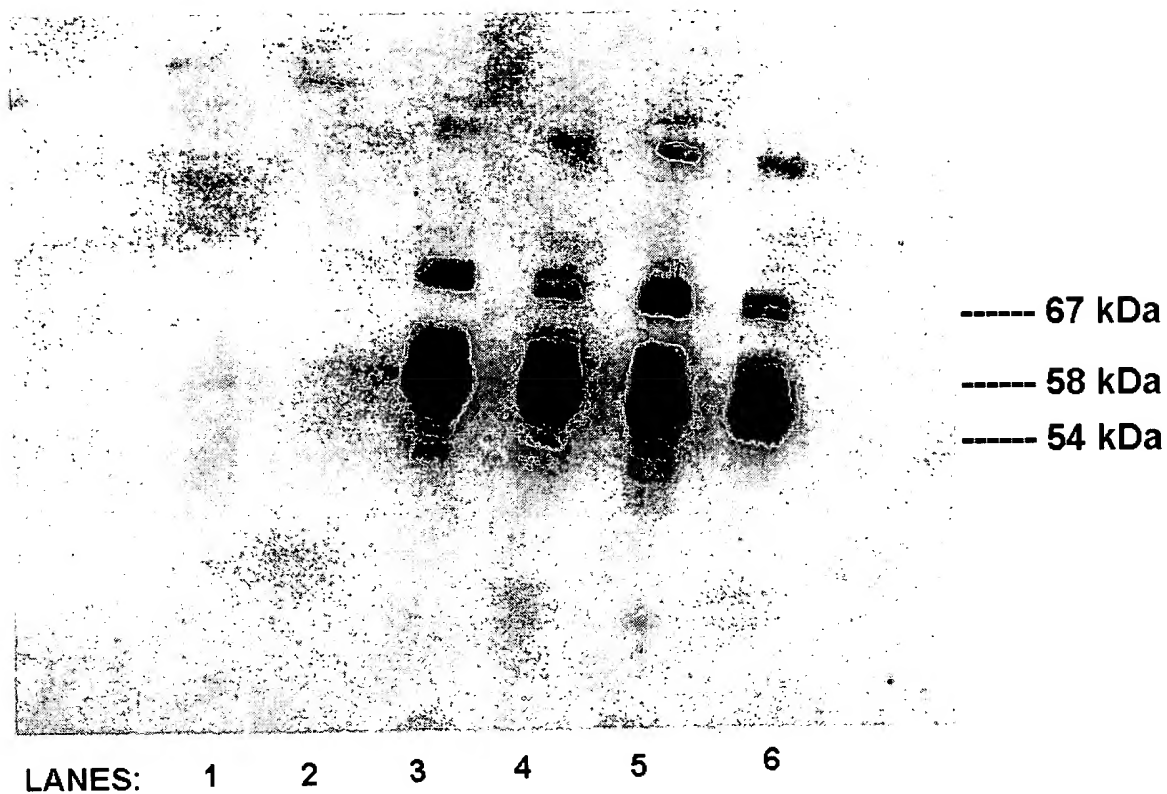### EFFECT OF CA-PS-POOL II ON ESTROGEN RESPONSIVE GROWTH IN SERUM FREE MEDIUM

LEGEND: Open circles = $- E_2$

Closed circles = $+ E_2$

FIGURE 58

WESTERN ANALYSIS OF CBG (POOL I) AND

SHBG (POOL II) PREPARATION WITH ANTI-54 kDa

------ 67 kDa

------ 58 kDa

------ 54 kDa

LANES: 1   2   3   4   5   6

1 = CBG PREPARATION # 5

2 = CBG PREPARATION # 6

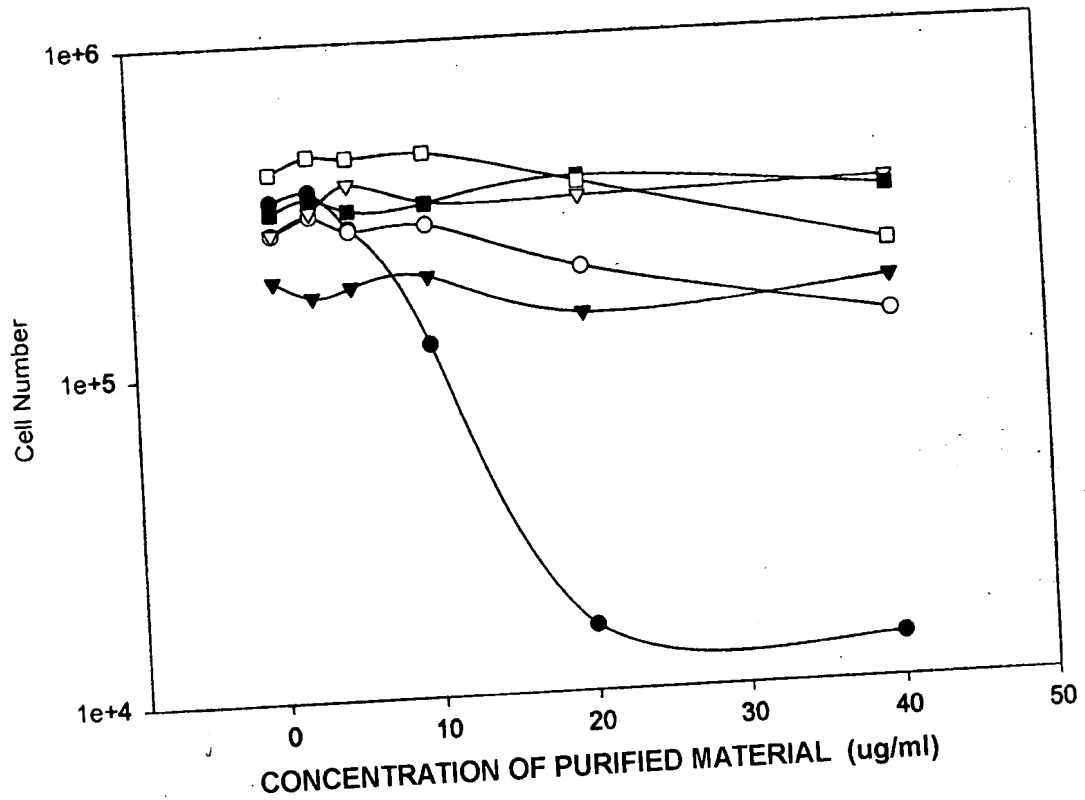3 = SHBG PREPARATION # 5.1

4 = SHBG PREPARATION # 5.2

5 = SHBG PREPARATION # 6.1

6 = SHBG PREPARATION # 6.2

ANTIBODY = RABBIT ANTI-54 kDa 1:5000 DILUTION

FIGURE 59

EFFECT OF ANTI-54kDa ANTISERUM ON MTW9/PL2 CELLS GROWN IN THE PRESENCE OF CA-PS-POOL II

LEGEND:
- —●— No antibody
- —○— Antibody 1:5000
- —▼— Antibody 1:1000
- —▽— Antibody 1:500
- —■— Antibody 1:100
- —□— Antibody 1:50

FIGURE 60

WESTERN BLOT OF COMMERCIAL PREPARATIONS OF HORSE IgA, IgG AND IgM WITH THE ANTI-54 kDa ANTIBODY
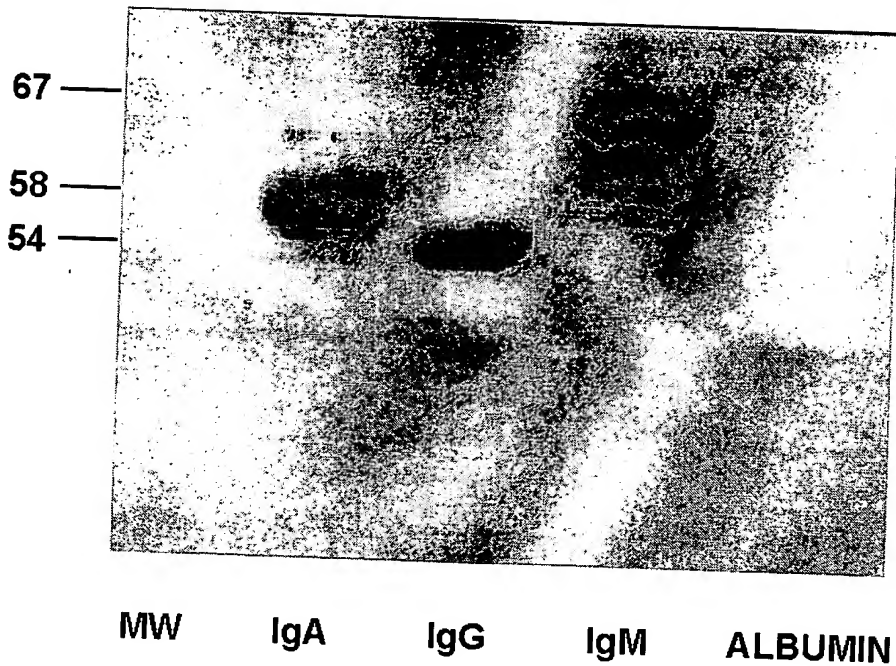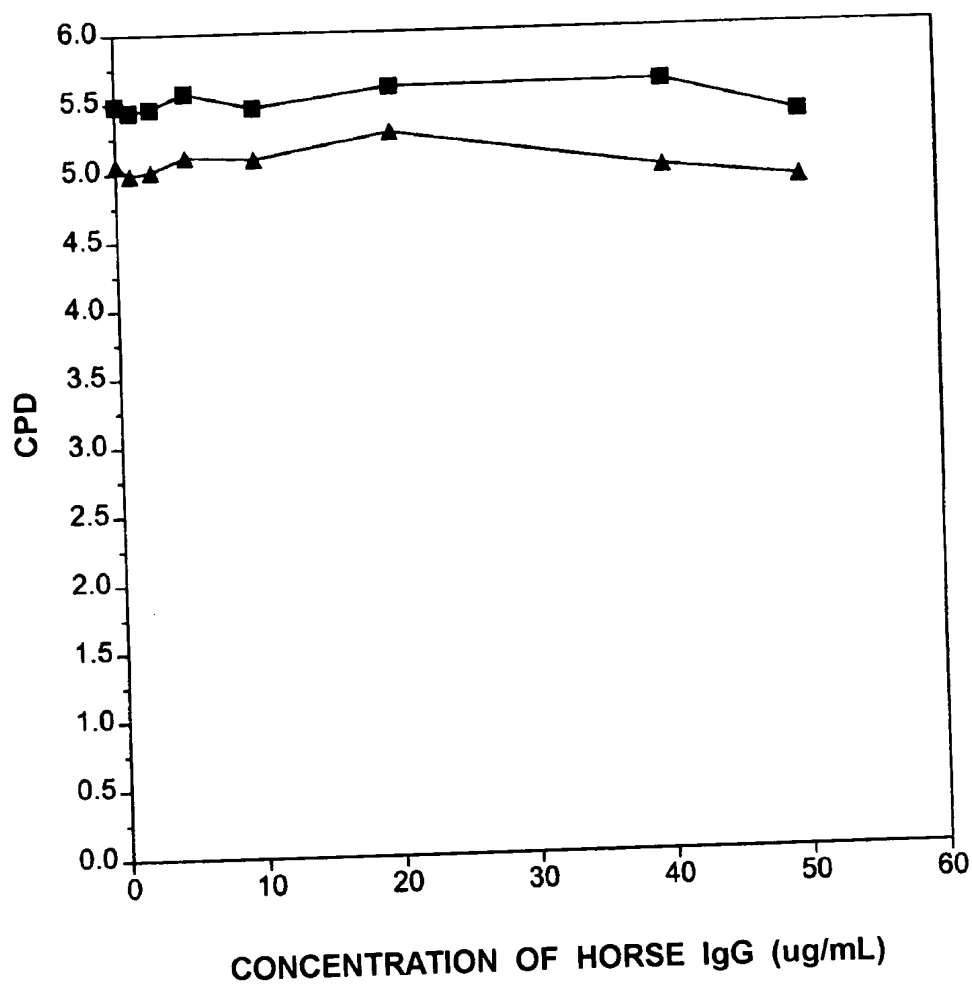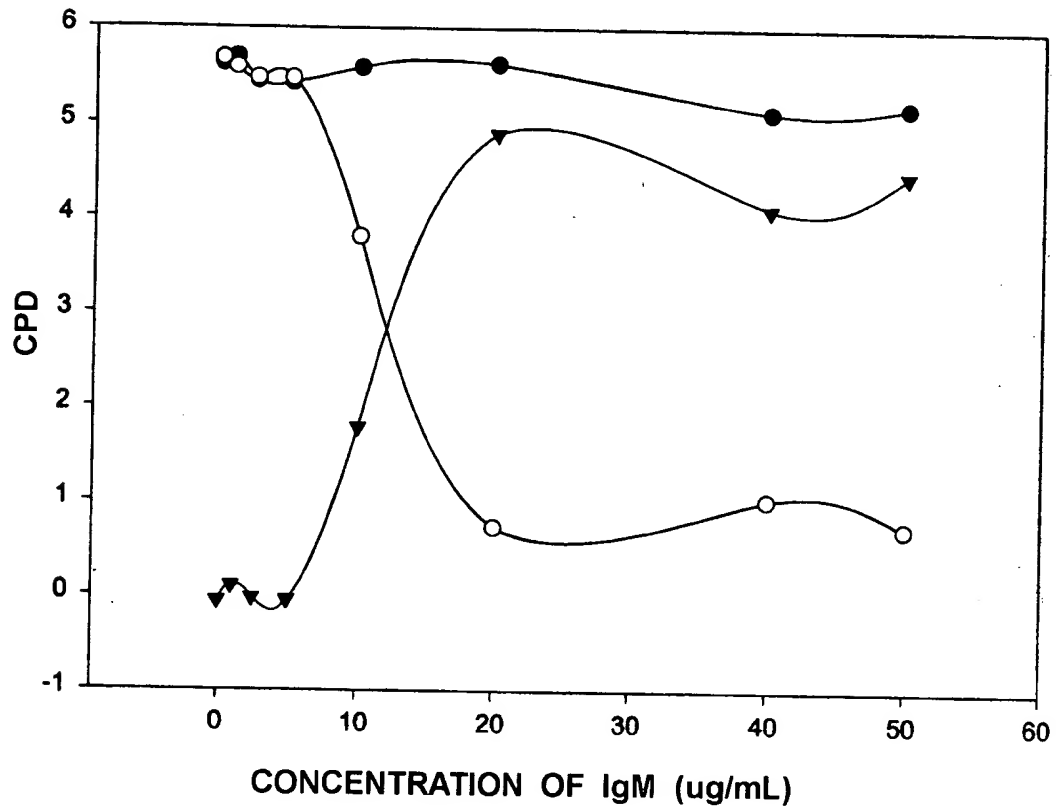
MkDa

67 —

58 —
54 —

MW   IgA   IgG   IgM   ALBUMIN

FIGURE 61

EFFECT OF COMMERCIALLY PURIFIED HORSE IgG

ON MTW9/PL2 CELL GROWTH IN 2.5% CDE-HORSE SERUM

CONCENTRATION OF HORSE IgG (ug/mL)

LEGEND: —■— plus E_2

—▲— minus E_2

FIGURE 62

EFFECT OF HORSE IgM ON GROWTH OF THE
MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM $\pm$ E$_2$
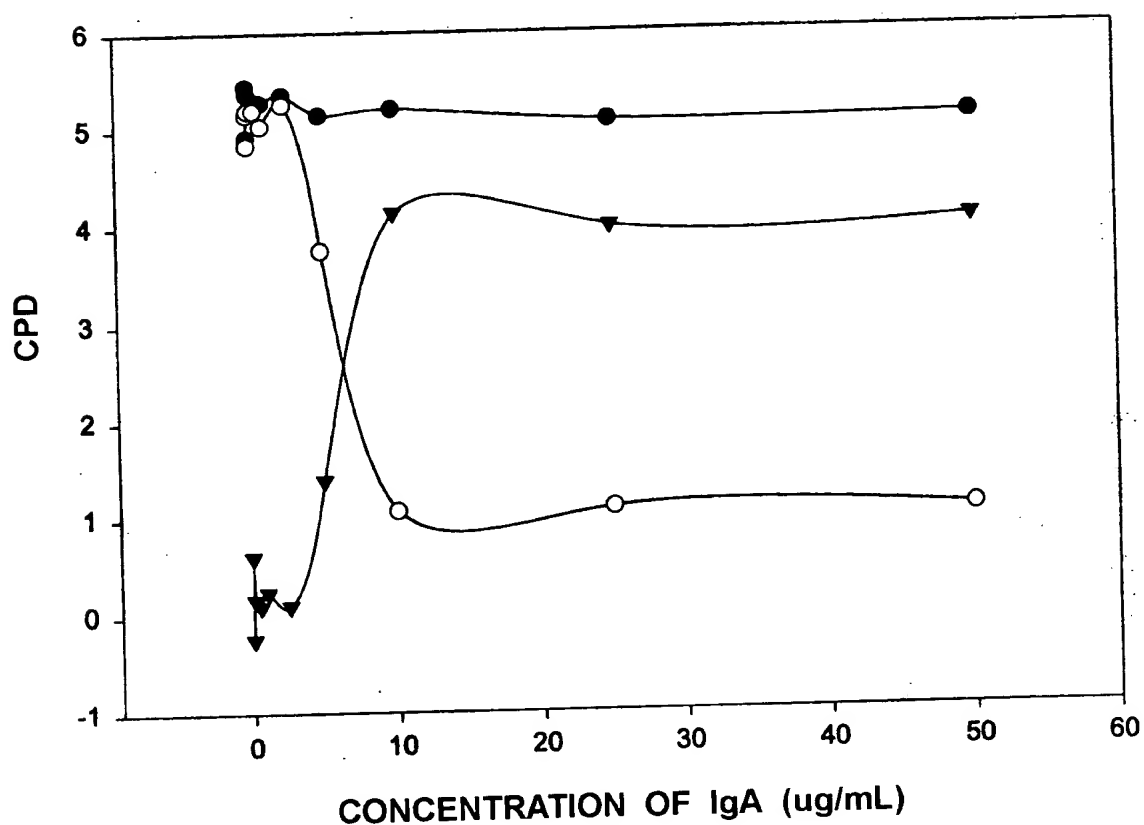

LEGEND:

—●— = + E$_2$

—○— = – E$_2$

—▼— = Estrogenic effect

FIGURE 63

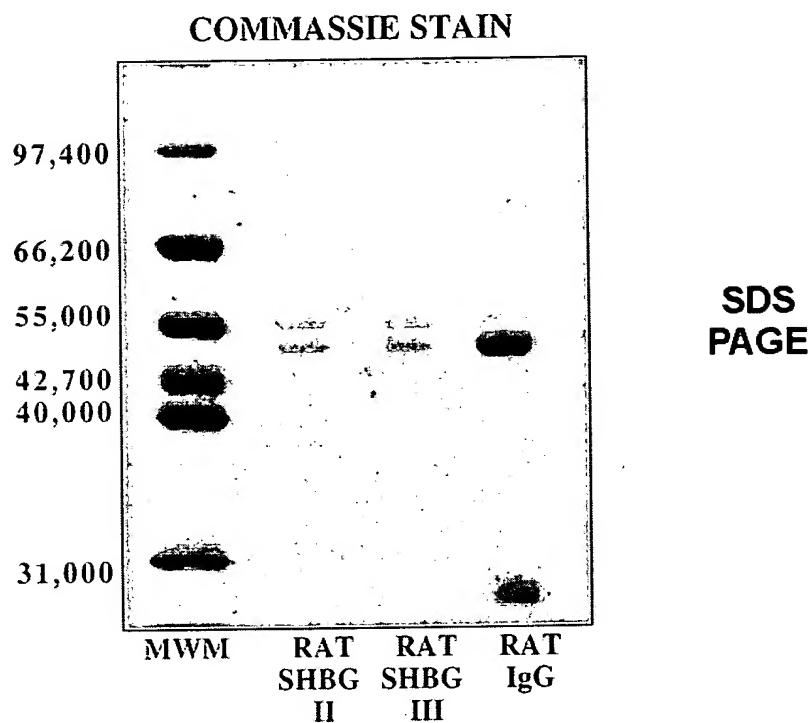## EFFECT OF HORSE IgA ON GROWTH OF THE MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM $\pm E_2$
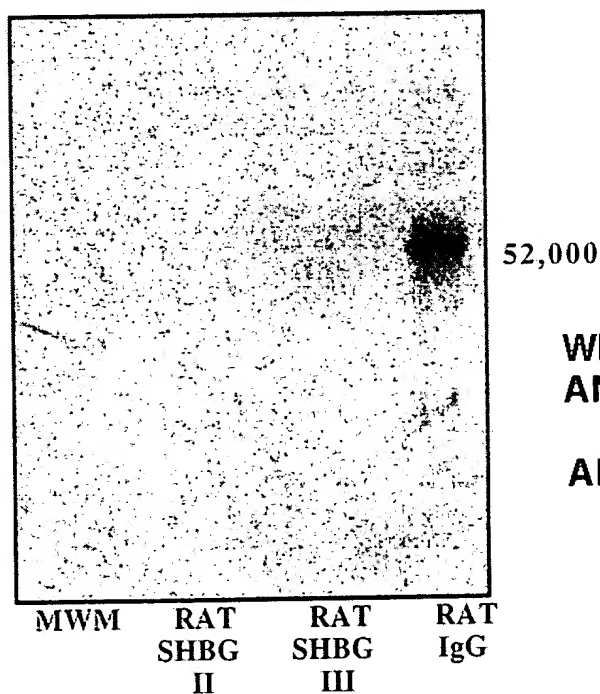

LEGEND:

—●— = $+ E_2$

—○— = $- E_2$

—▼— = Estrogenic effect

FIGURE 64

SDS PAGE AND WESTERN ANALYSIS OF RAT "SHBG-LIKE" PREPARATIONS

COMMASSIE STAIN

SDS
PAGE

97,400
66,200
55,000
42,700
40,000
31,000

MWM · RAT SHBG II · RAT SHBG III · RAT IgG

WESTERN BLOT. ANTI IgG

52,000

WESTERN
ANALYSIS
WITH
ANTI-RAT
IgG

MWM · RAT SHBG II · RAT SHBG III · RAT IgG
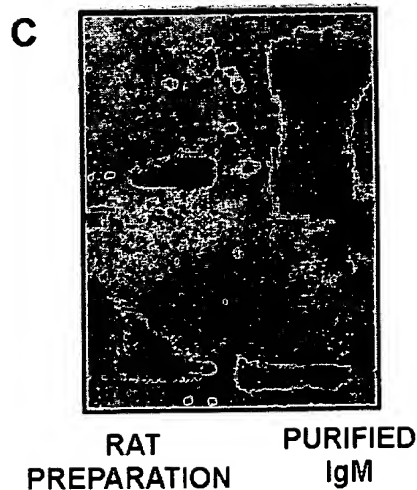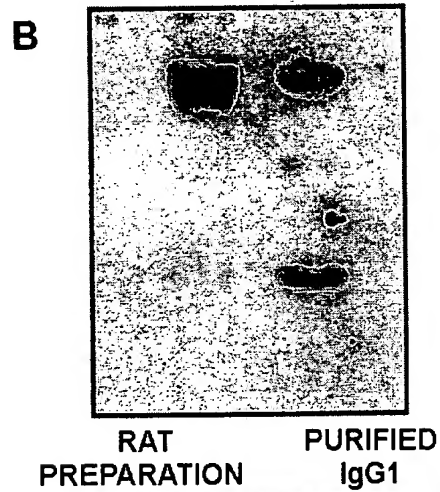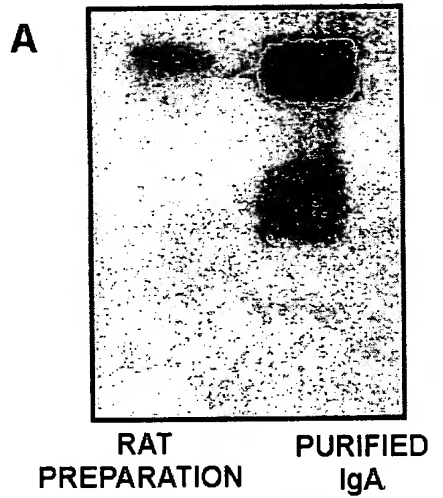
FIGURE 65

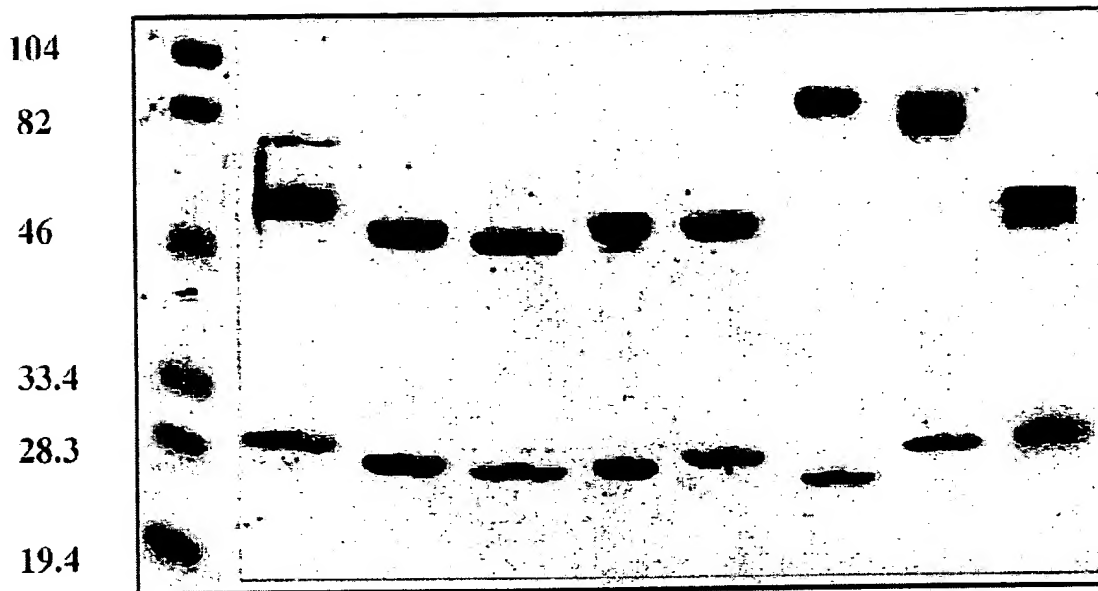## CROSSREACTION OF THE PURIFIED RAT "SHBG-LIKE" PROTEINS WITH ANTI- IgA, IgG1 AND IgM MONOCLONAL ANTIBODIES

A

RAT   PURIFIED
PREPARATION   IgA

B

RAT   PURIFIED
PREPARATION   IgG1

C

RAT   PURIFIED
PREPARATION   IgM

FIGURE 66

SDS PAGE (A) AND WESTERN ANALYSIS (B)
WITH ANTI-SHBG AND RAT Ig'S

RAT Igs COMMASSIE STAINED

A KDa

104
82
46
33.4
28.3
19.4
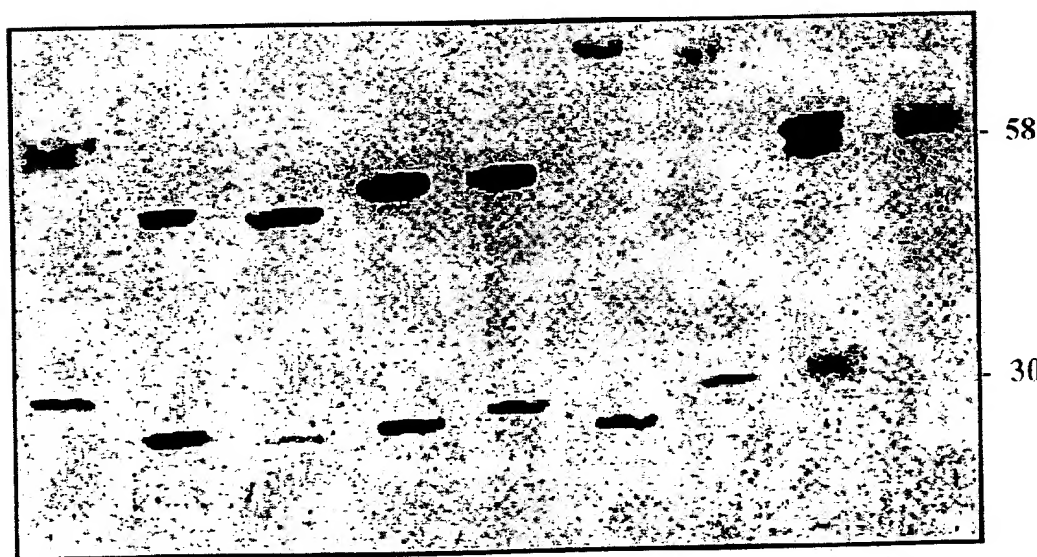
MW   IgA   IgG1   IgG2a   IgG2b   IgG2c   IgE   IgM   RP

RAT Igs WESTERN BLOT. ANTI SHBG ANTIBODY   KDa

B

58
30
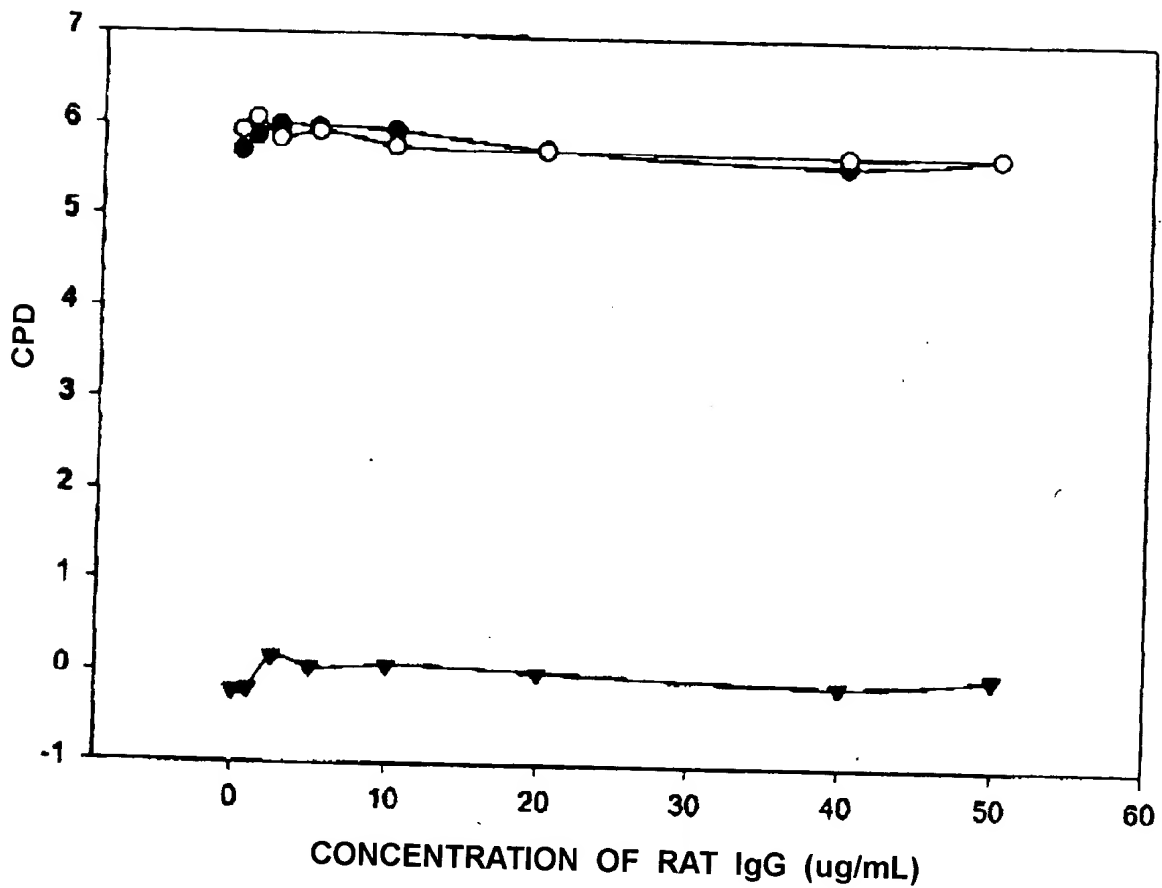
IgA   IgG1   IgG2a   IgG2b   IgG2c   IgE   IgM   HP   RP

FIGURE 67

EFFECT OF RAT IgG ON MTW9/PL2 CELL
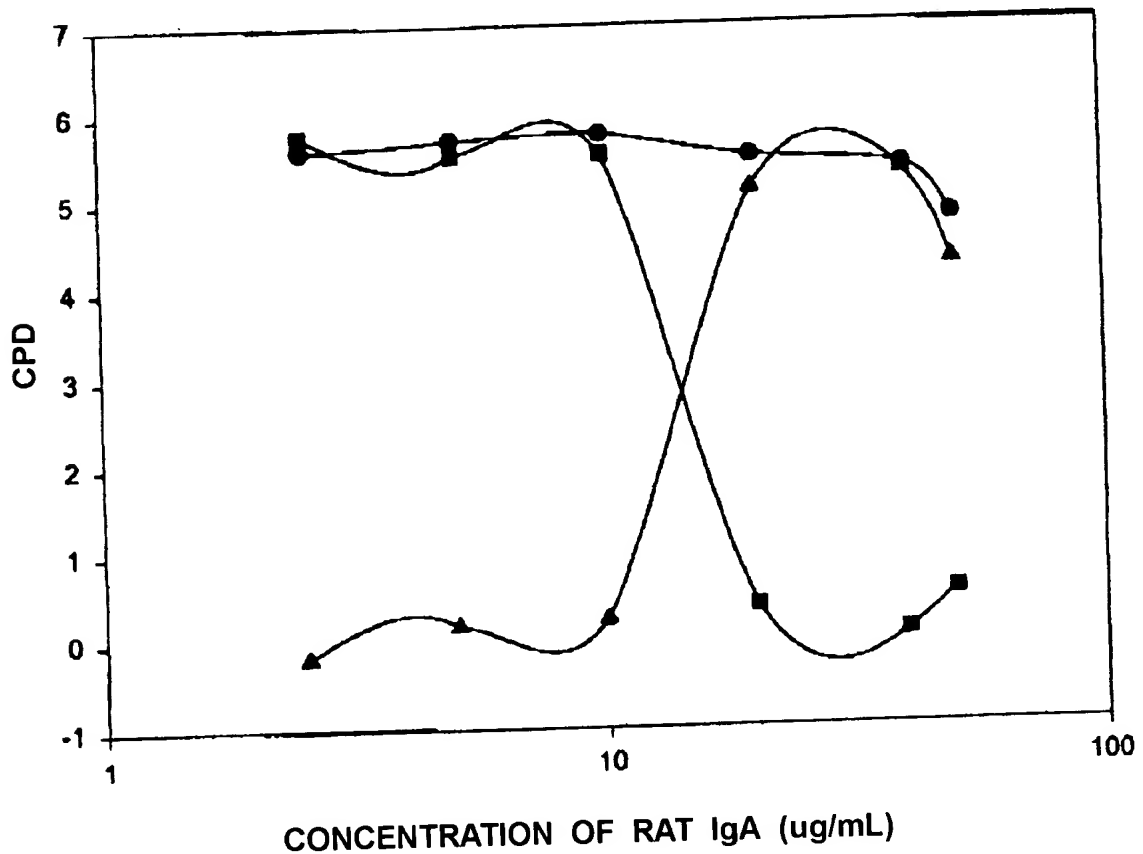## GROWTH IN 2.5% CDE RAT SERUM

LEGEND:

Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 68

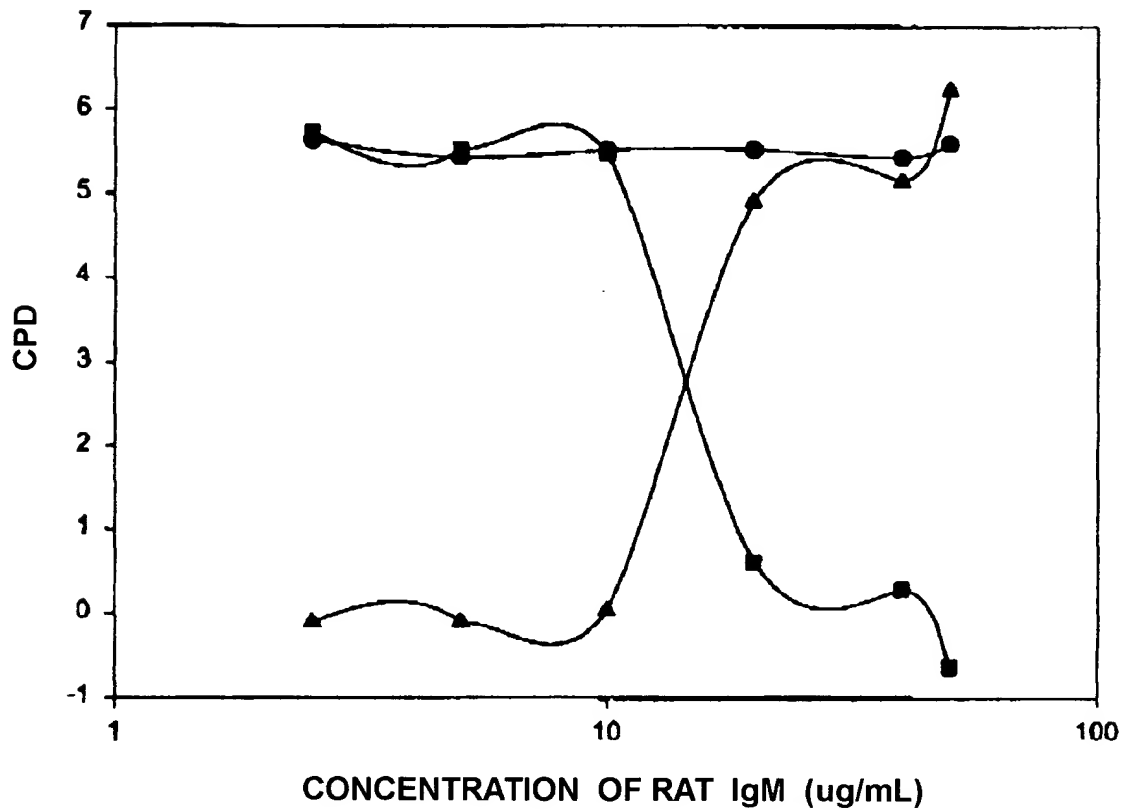## EFFECT OF RAT IgA ON MTW9/PL2 CELL GROWTH IN 2.5% CDE RAT SERUM

LEGEND:

Closed circles = + E_2

Closed squares = − E_2

Closed triangles = Estrogenic effect

FIGURE 69

EFFECT OF RAT IgM ON MTW9/PL2 CELL
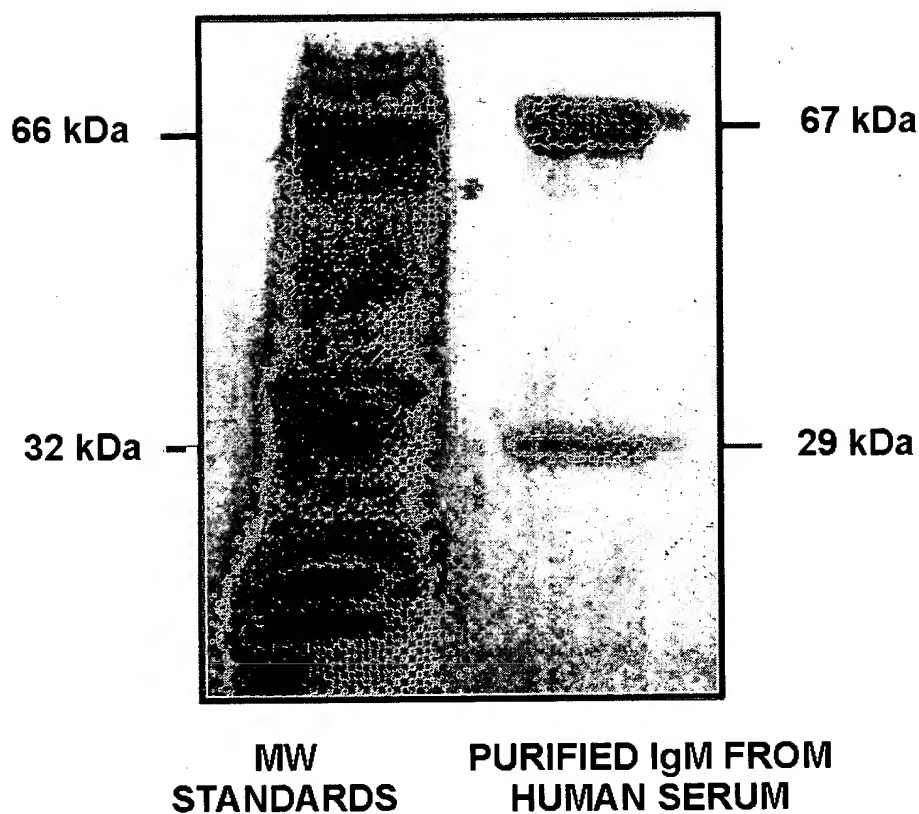
GROWTH IN 2.5% CDE RAT SERUM

LEGEND:

Closed squares = − E$_2$

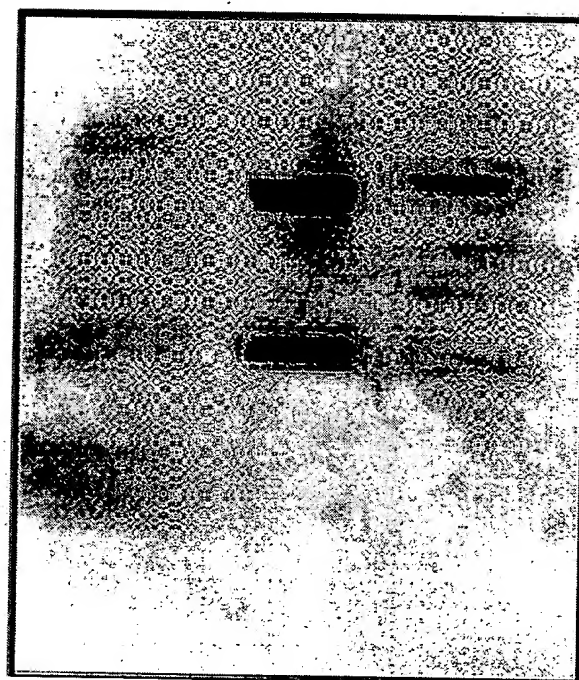**Closed circles = + E$_2$**

Closed triangles = Estrogenic effect

FIGURE 70

ELUTION OF IgM FROM MANNAN
BINDING PROTEIN COLUMN

MW
STANDARDS

PURIFIED IgM FROM
HUMAN SERUM

FIGURE 71

IgM PURIFICATION FROM

PLASMA BY JACALIN

MW   HUMAN   PURIFIED
IgA   IgA

FIGURE 72

EFFECT OF IgM ISOLATED FROM HUMAN PLASMA ON MTW9/PL2 GROWTH IN SERUM-FREE CONDITIONS
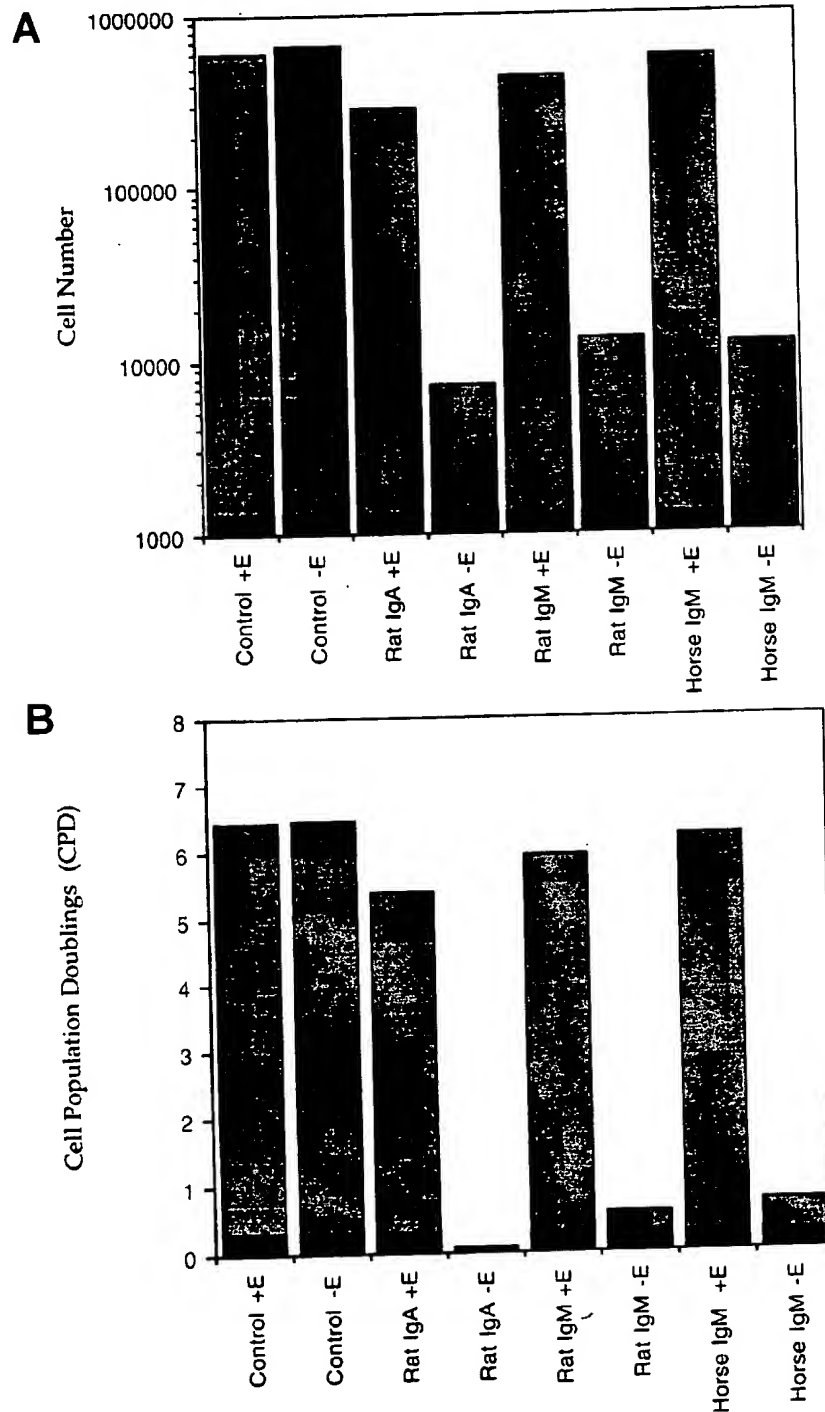

HUMAN PLASMA IgM (ug/mL)

LEGEND:
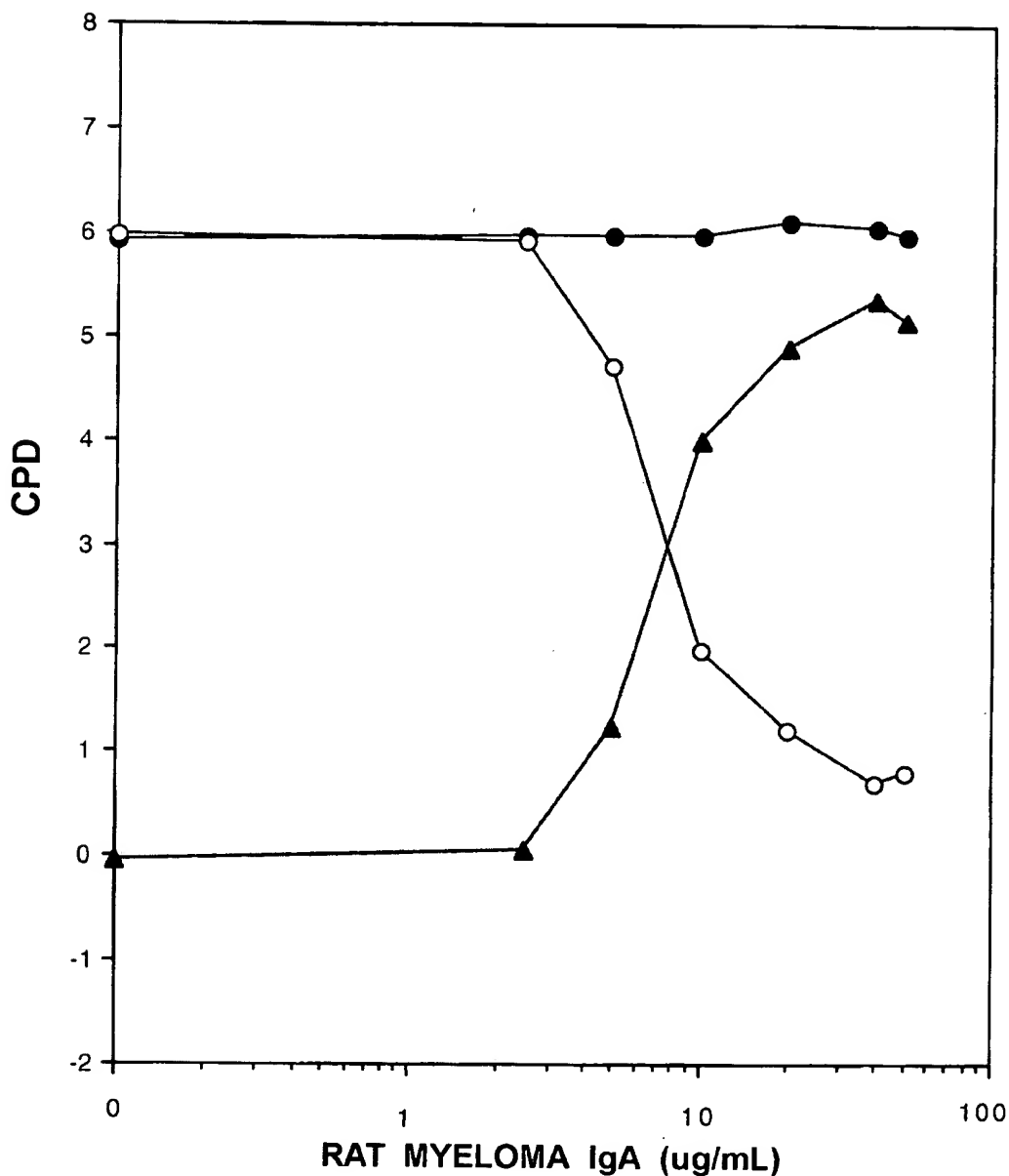
—●— = + E_2

—+— = − E_2
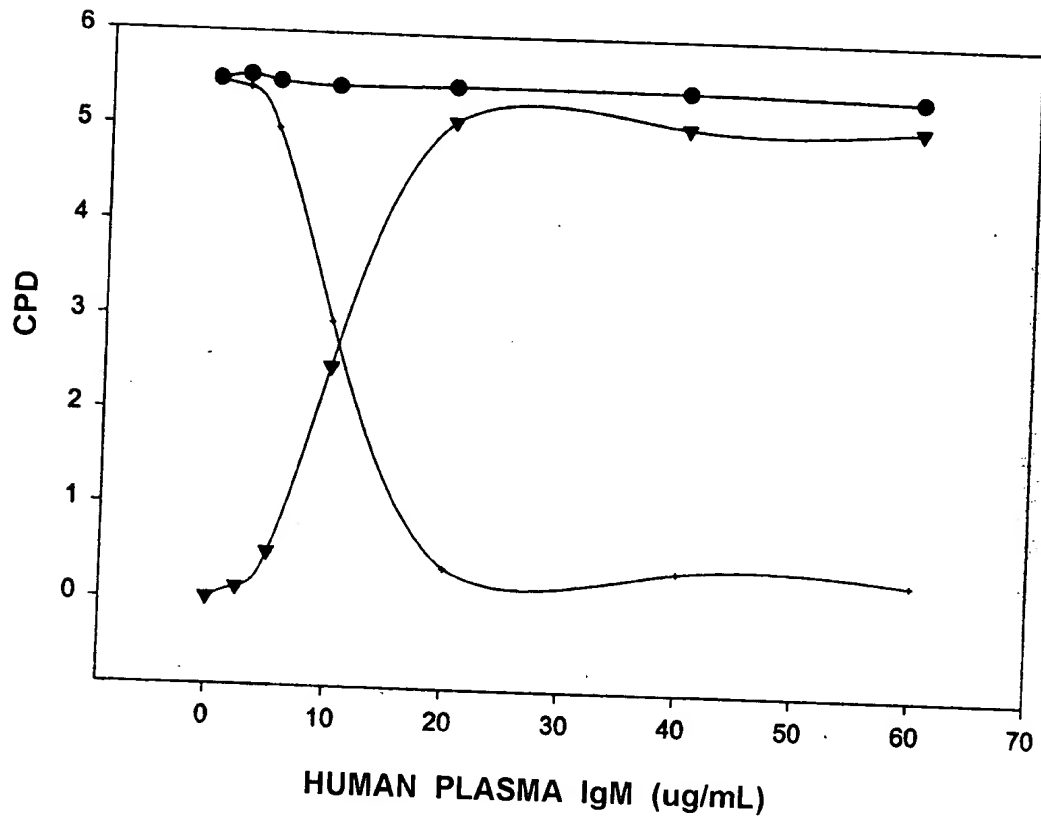
—▼— = Estrogenic effect

FIGURE 73

THE EFFECT OF VARIOUS IgA AND IgM PREPARATIONS ON MTW9/PL2 CELLS GROWN IN SERUM-FREE MEDIUM

FIGURE 74

RAT MYELOMA IgA TITRATION ON GH$_1$ CELLS GROWN IN SERUM-FREE CONDITIONS

LEGEND:

Closed circles = + E$_2$

Open circles = − E$_2$

Closed triangles = Estrogenic effect

FIGURE 75

HUMAN PLASMA IgA TITRATION ON GH$_1$ CELLS GROWN IN SERUM-FREE CONDITIONS

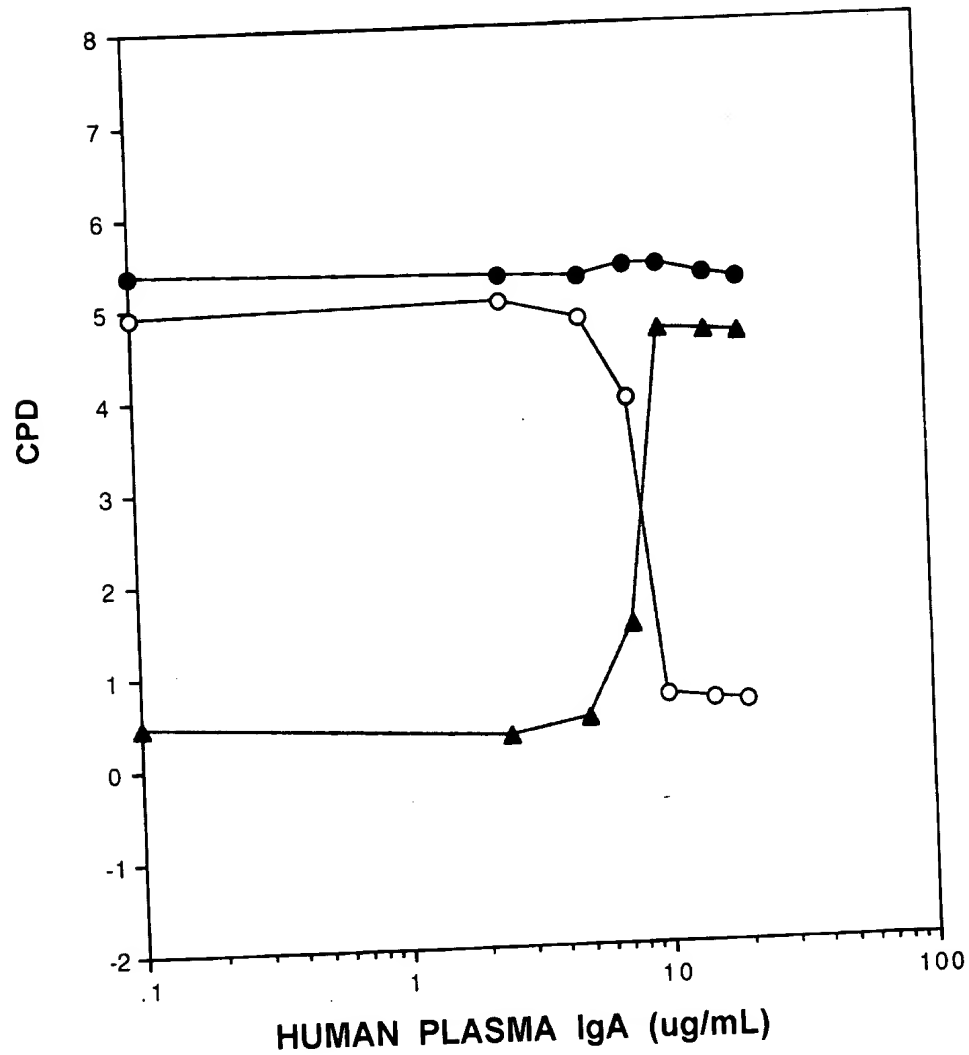

LEGEND:

Closed circles = + E$_2$

Open circles = – E$_2$

Closed triangles = Estrogenic effect

FIGURE 76

HUMAN PLASMA IgM TITRATION ON GH_1 CELLS
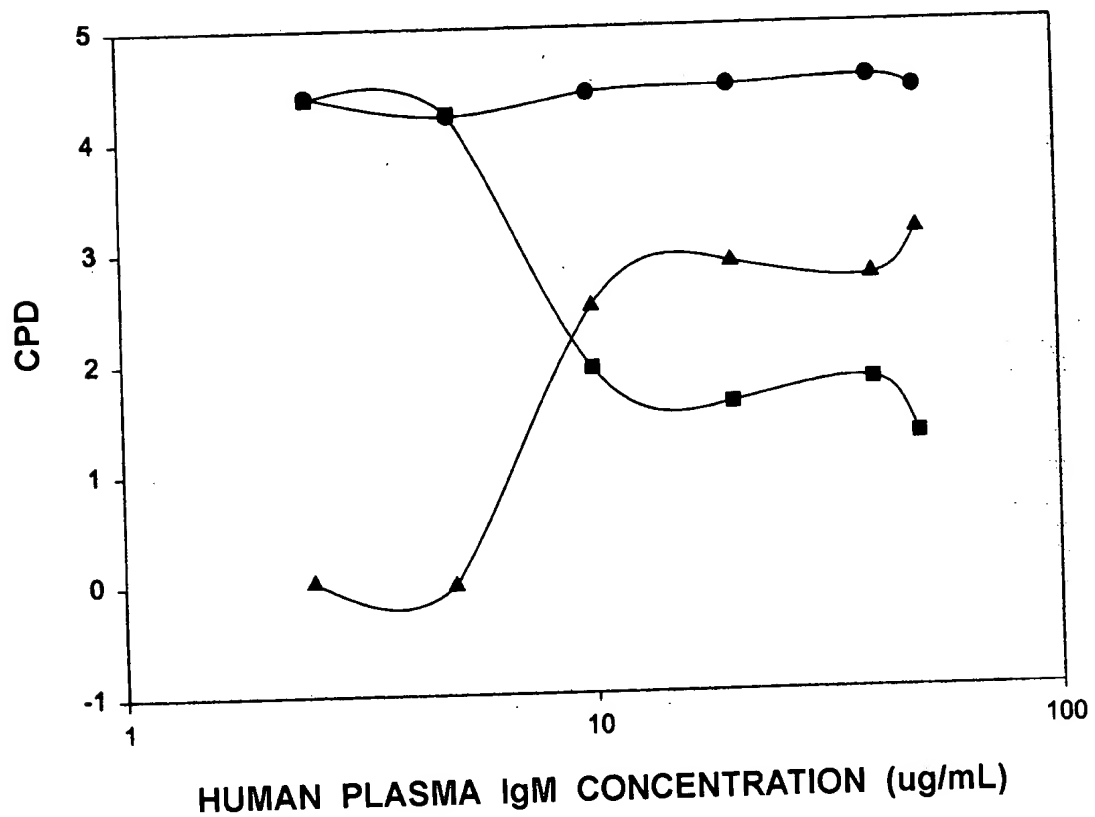## GROWN IN SERUM-FREE CONDITIONS

HUMAN PLASMA IgM CONCENTRATION (ug/mL)

LEGEND:

—●— = + E_2

—■— = − E_2

—▲— = Estrogenic effect

FIGURE 77

EFFECT OF HUMAN SECRETORY IgA ON
GH$_1$ CELLS GROWN IN SERUM-FREE CONDITIONS
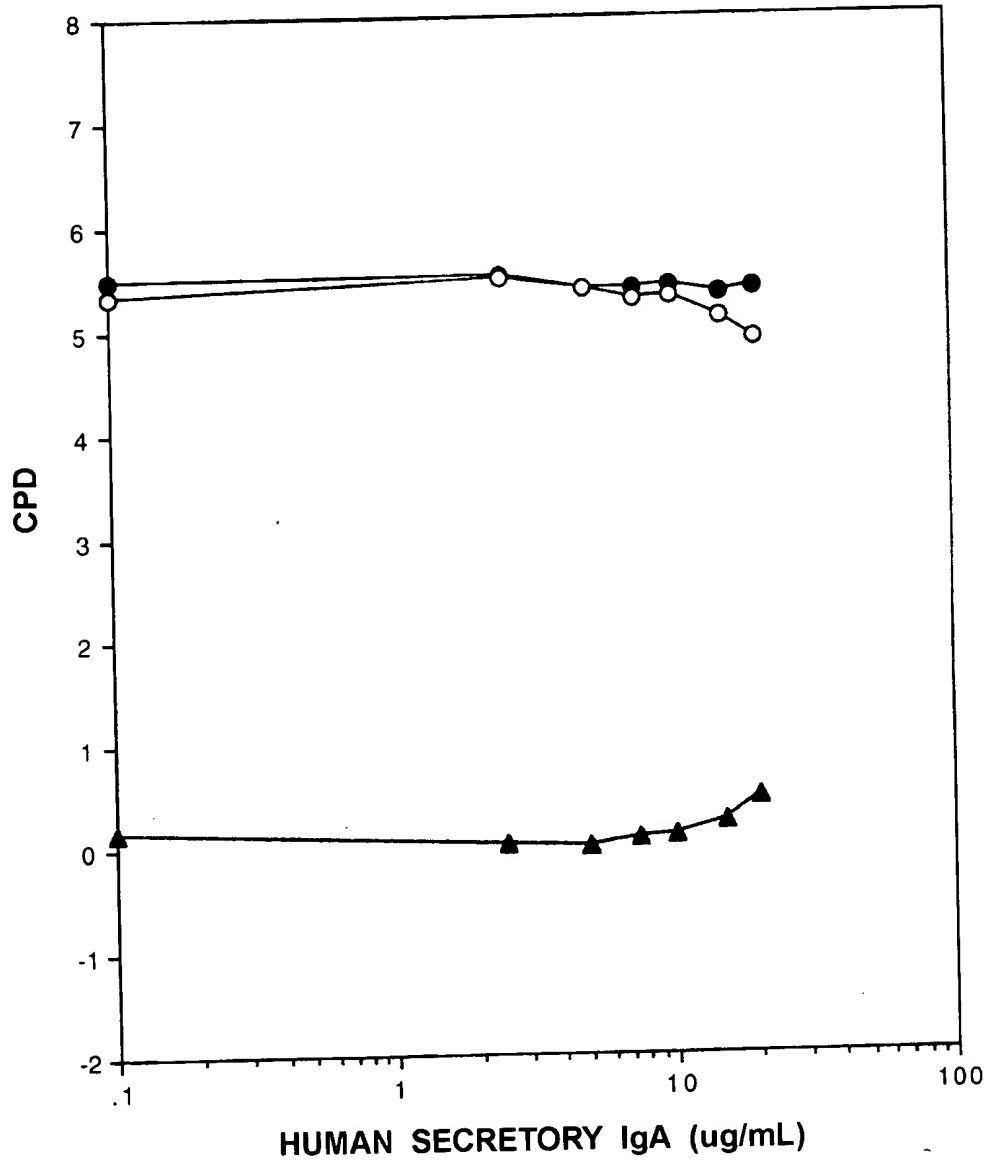
LEGEND:
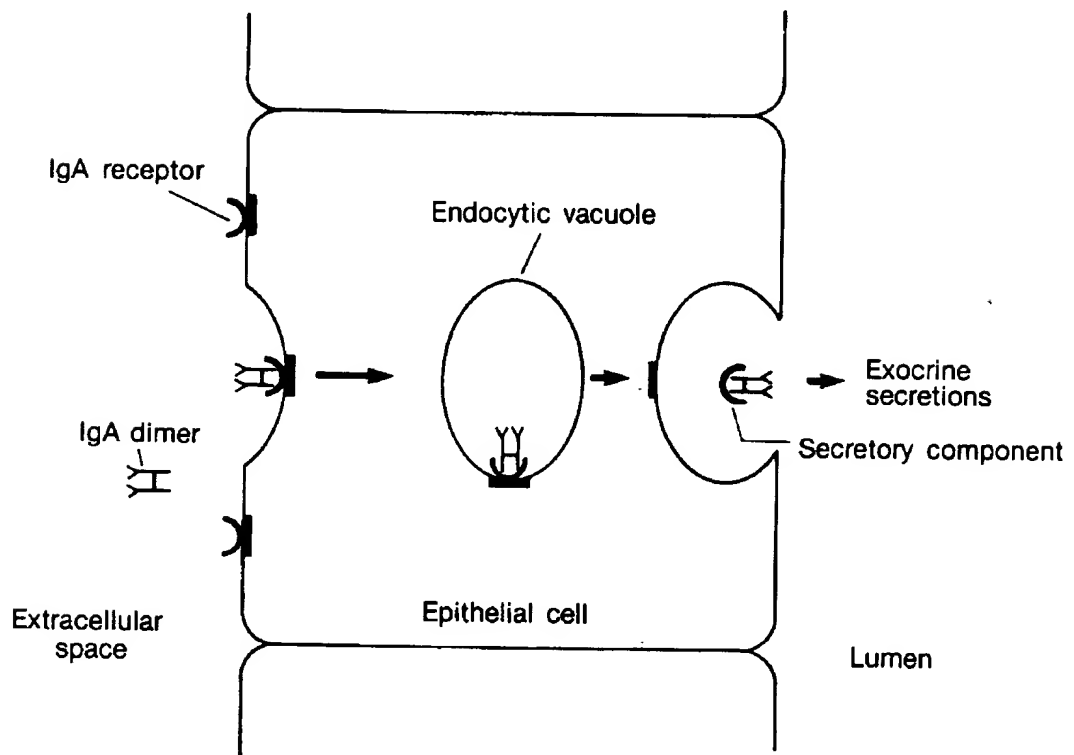
Closed circles = + E$_2$

Open circles = − E$_2$

Closed triangles = Estrogenic effect

FIGURE 78

MECHANISM OF TRANSCYTOSIS OF IgA AND IgM
BY MUCOSAL EPITHELIAL CELLS

IgA receptor

Endocytic vacuole

IgA dimer

Exocrine
secretions

Secretory component

Extracellular
space

Epithelial cell

Lumen

FIGURE 79

ESSENTIAL STRUCTURES OF HUMAN
PLASMA AND SECRETORY IgA
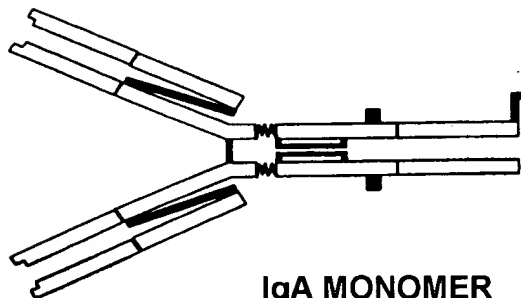
**IgA MONOMER
(INACTIVE)**

J CHAIN

**IgA DIMER WITH
ATTACHED J CHAIN (ACTIVE)**

**SECRETORY PIECE OR
SECRETORY COMPONENT
(80% POLY-IgR)**

**SECRETORY IgA SHOWING J CHAIN
AND SECRETORY COMPONENT (INACTIVE)**

FIGURE 80

EFFECT OF RAT MYELOMA IgA ON GH$_3$
CELLS GROWN IN SERUM-FREE MEDIUM

LEGEND:

Closed circles = + E$_2$

Open circles = − E$_2$

Closed triangles = Estrogenic effect

FIGURE 81
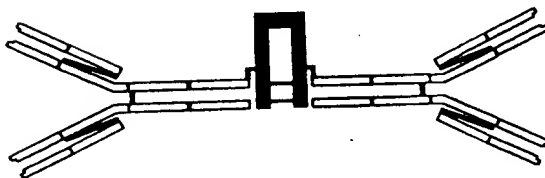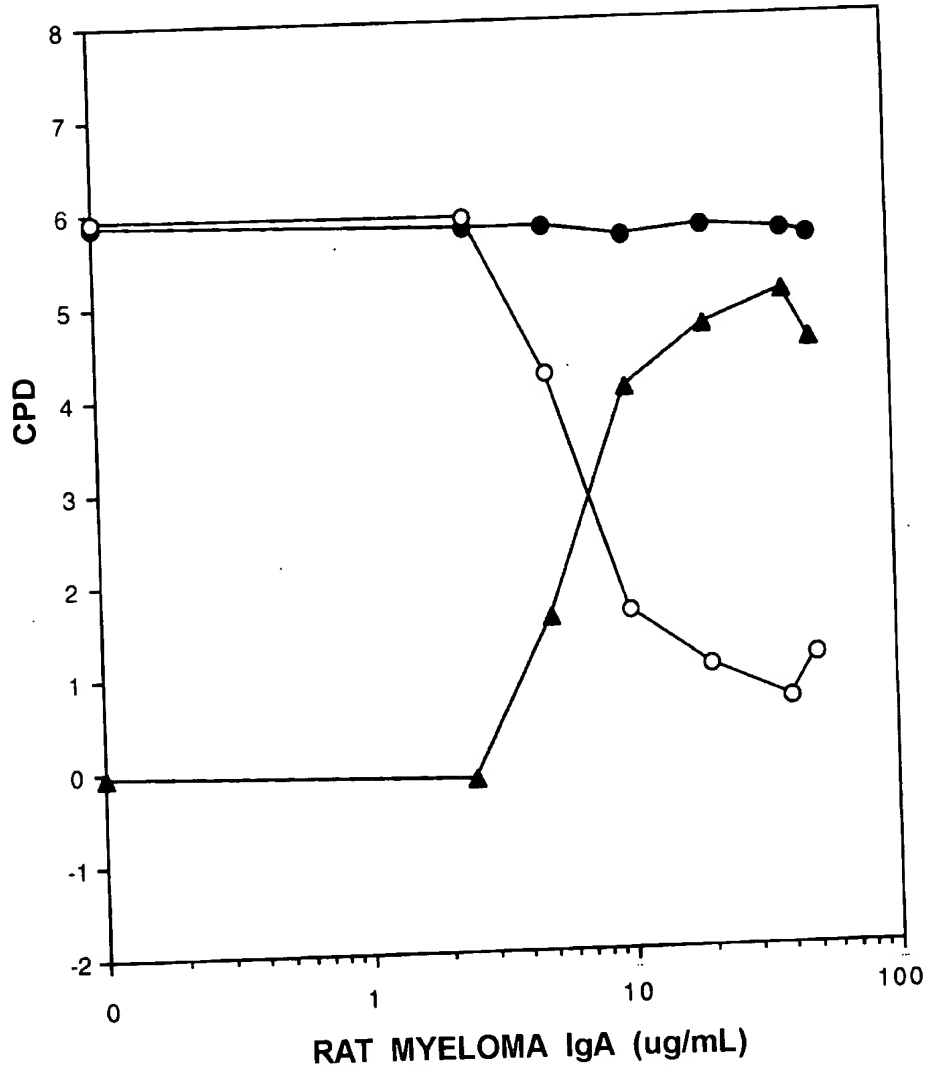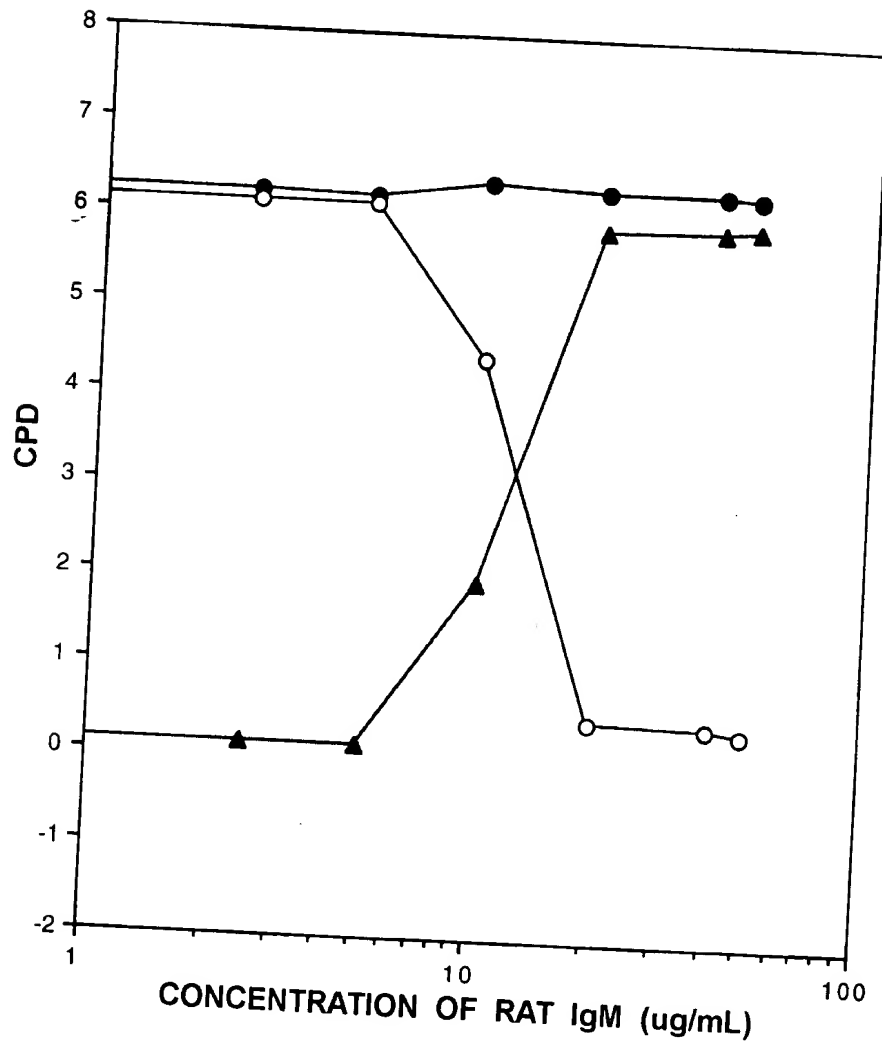
EFFECT OF RAT IgM ON GH$_3$ CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND:

—●— = + E$_2$

—○— = − E$_2$

—▲— = Estrogenic effect

FIGURE 82

EFFECT OF HUMAN PLASMA IgA ON GH$_3$
CELL GROWTH IN SERUM-FREE MEDIUM
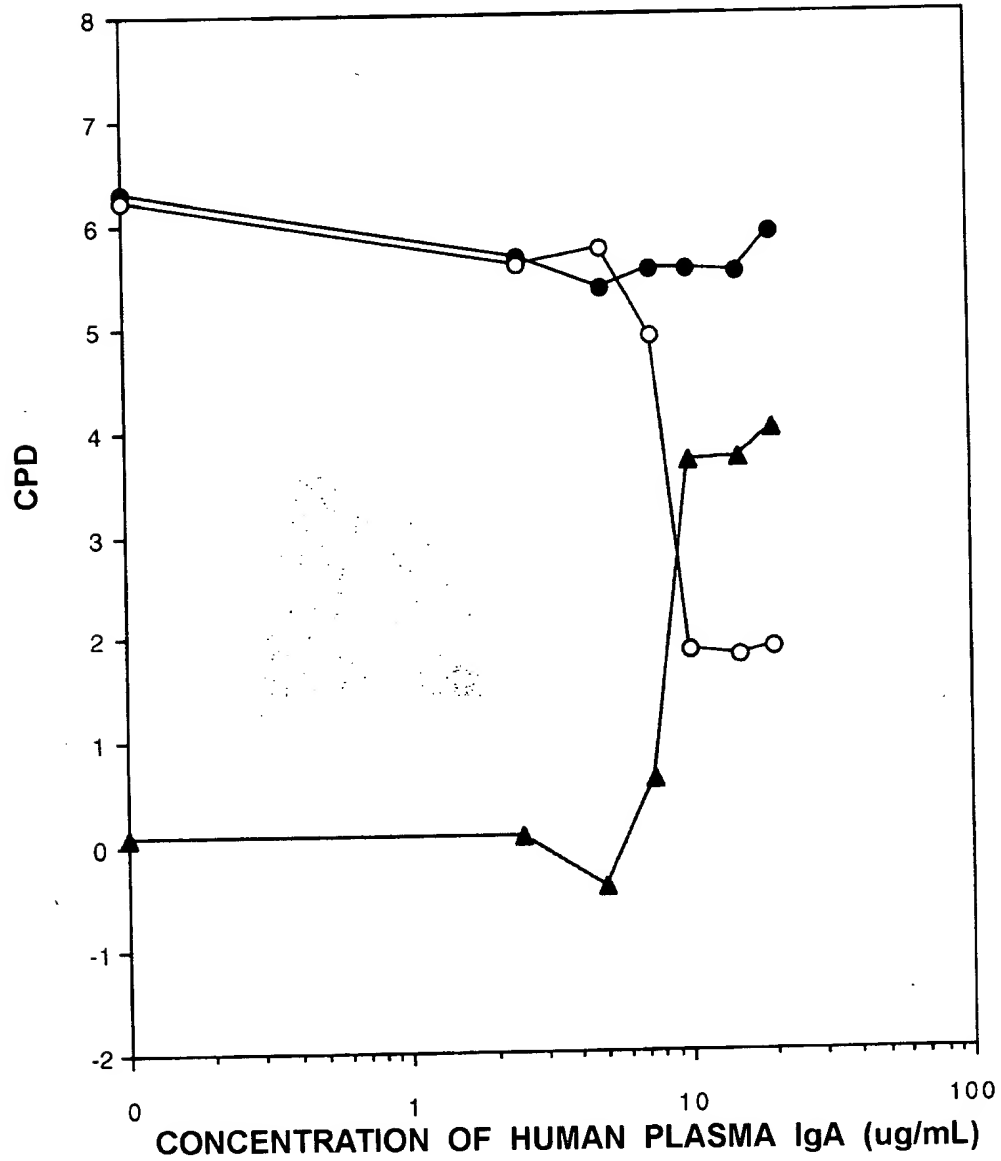
LEGEND:

Closed circles = + E$_2$

Open circles = – E$_2$

Closed triangles = Estrogenic effect

FIGURE 83

EFFECT OF HUMAN PLASMA IgM ON GH $_3$
CELL GROWTH IN SERUM-FREE MEDIUM

CONCENTRATION OF HUMAN PLASMA IgA (ug/mL)

LEGEND:

$\bullet$ = + E $_2$

$\blacksquare$ = – E $_2$

$\blacktriangledown$ = Estrogenic effect

FIGURE 84

EFFECT OF HUMAN MILK SECRETORY IgA ON
GH$_3$ CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND:

Closed circles = + E$_2$

Open circles = – E$_2$

Closed triangles = Estrogenic effect

FIGURE 85

EFFECT OF RAT MYELOMA IgA ON GH$_4$
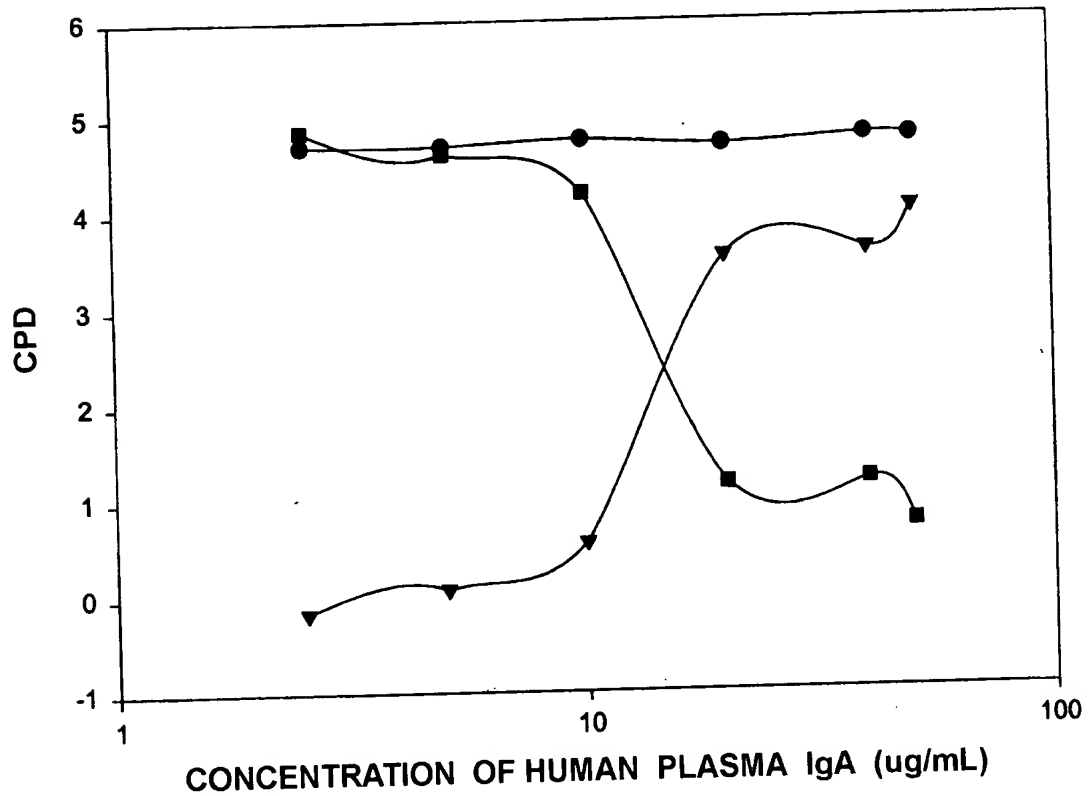### CELL GROWTH IN SERUM-FREE MEDIUM
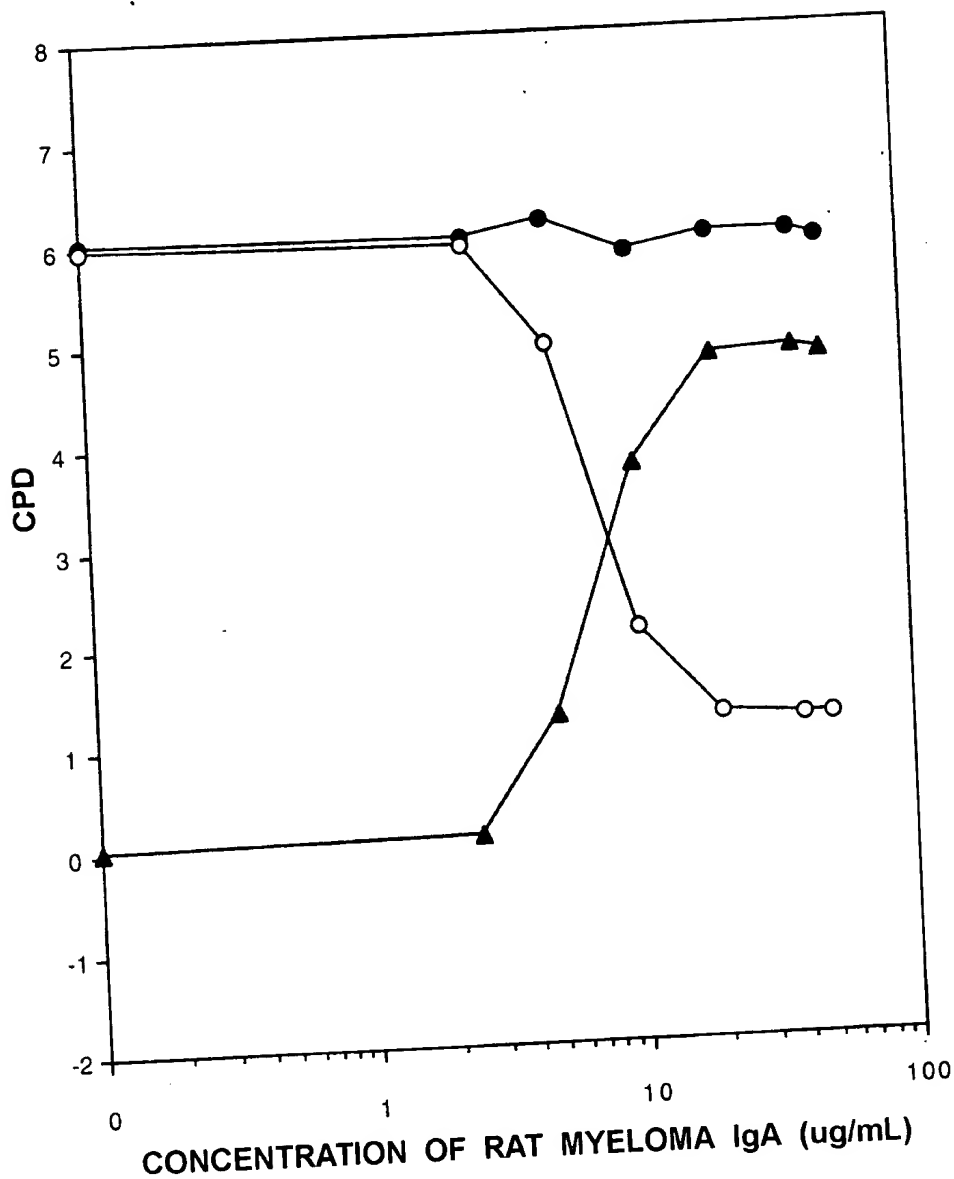
LEGEND:

Closed circles = + E$_2$

Open circles = − E$_2$

Closed triangles = Estrogenic effect

FIGURE 86

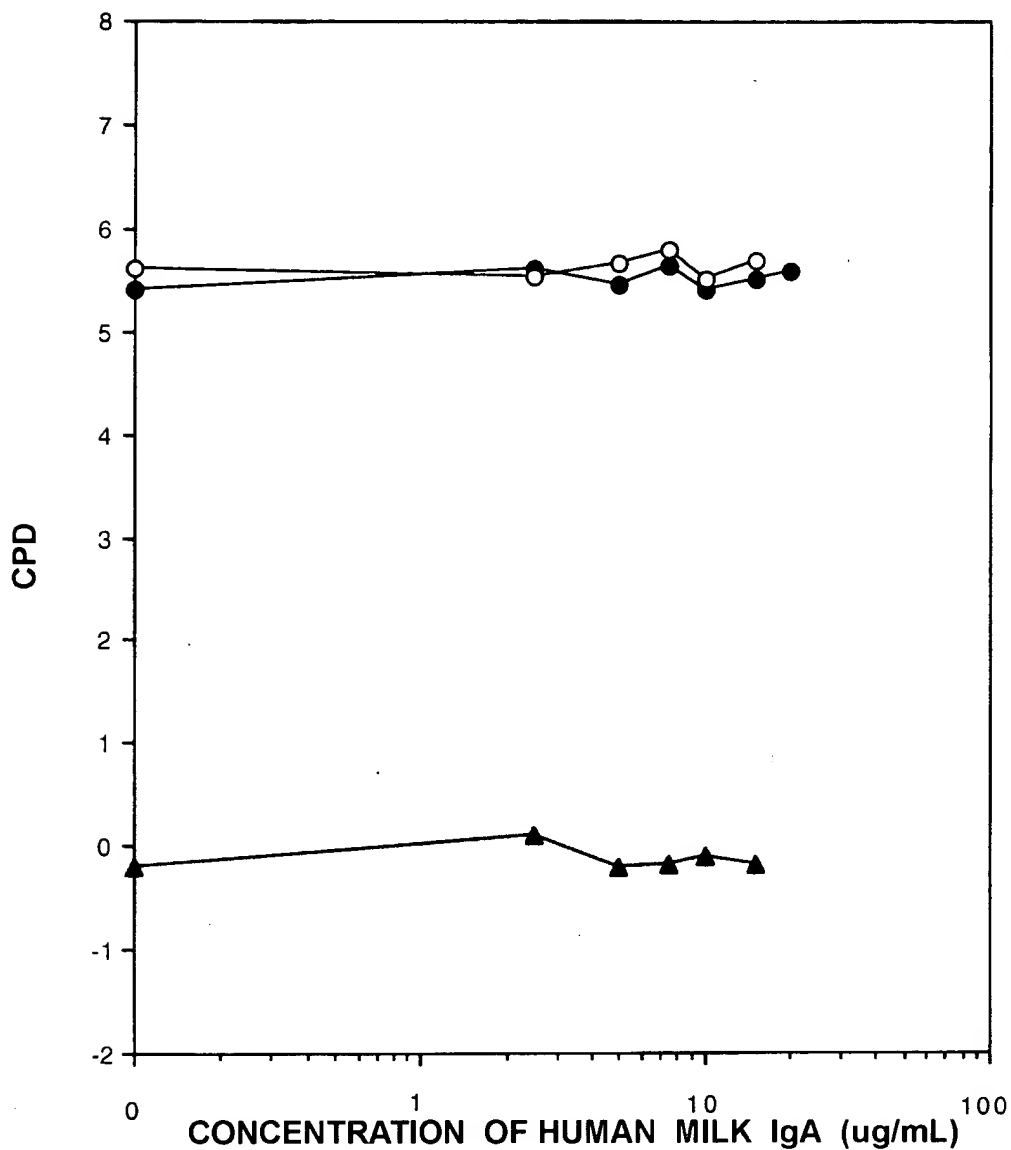## EFFECT OF RAT PLASMA IgM ON GH$_4$
CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND:

—○— = + E$_2$

—●— = − E$_2$
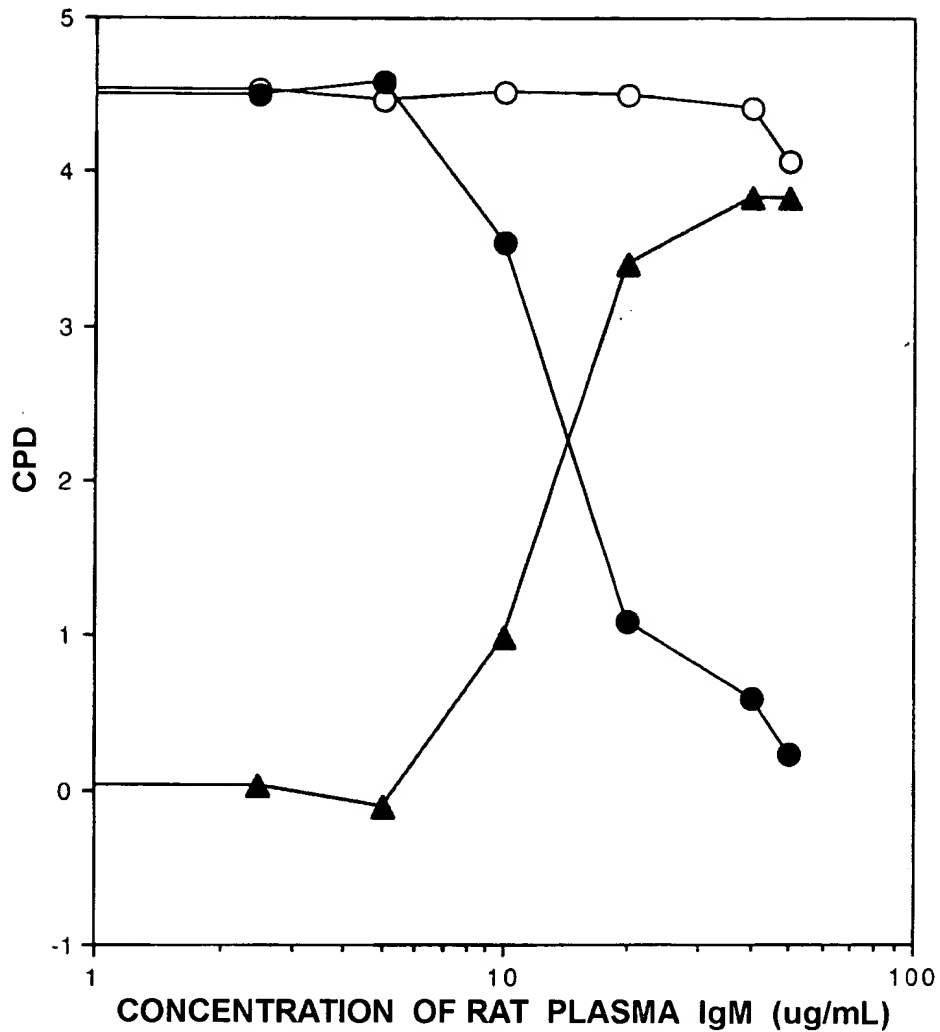
—▲— = Estrogenic effect

FIGURE 87

EFFECT OF HUMAN PLASMA IgA ON GH$_4$C$_1$ CELL GROWTH IN SERUM-FREE MEDIUM
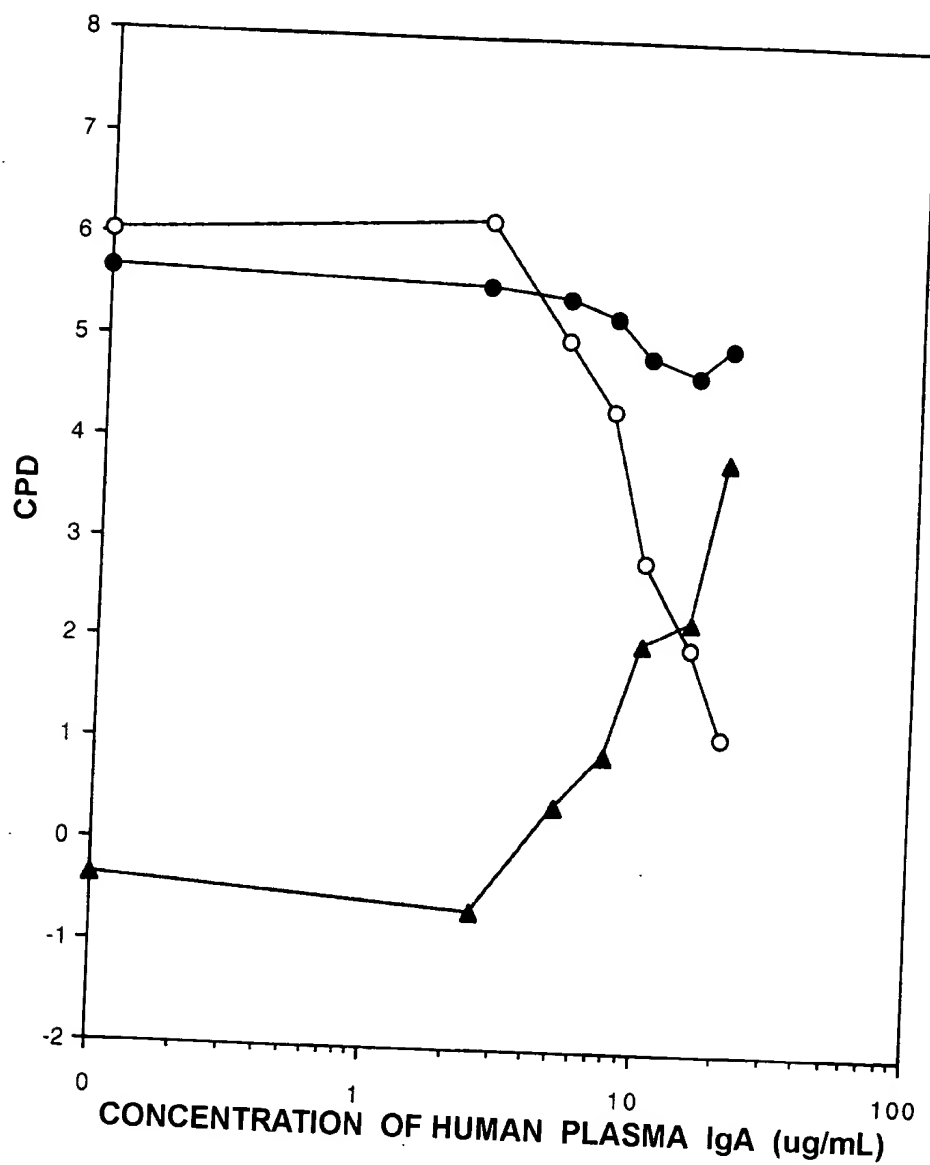
LEGEND:

Closed circles = + E$_2$

Open circles = − E$_2$

Closed triangles = Estrogenic effect

FIGURE 88

EFFECT OF HUMAN PLASMA IgM ON GH_4C_1 CELL GROWTH IN SERUM-FREE MEDIUM
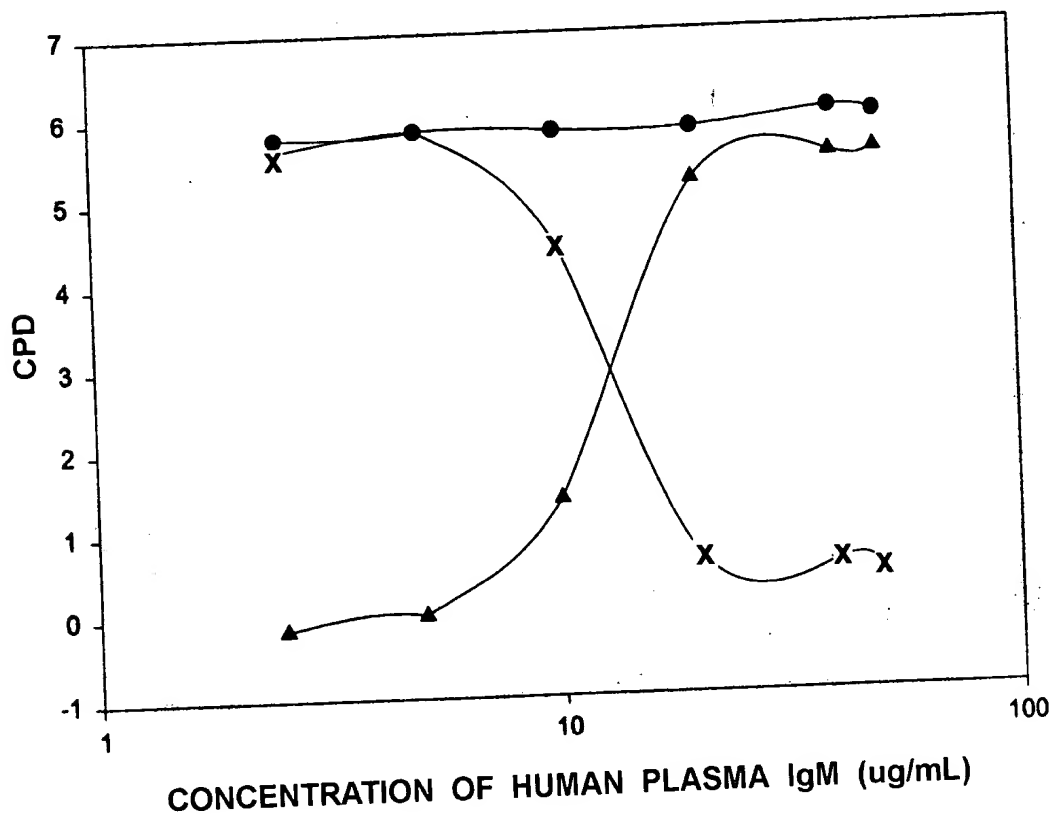
LEGEND:

$-\bullet- = + E_2$

$-X- = - E_2$

$-\blacktriangle- =$ Estrogenic effect

FIGURE 89

EFFECT OF HUMAN MILK SECRETORY IgA ON GH$_4$C$_1$ CELL GROWTH IN SERUM-FREE MEDIUM

CONCENTRATION OF HUMAN SECRETORY IgA (ug/mL)
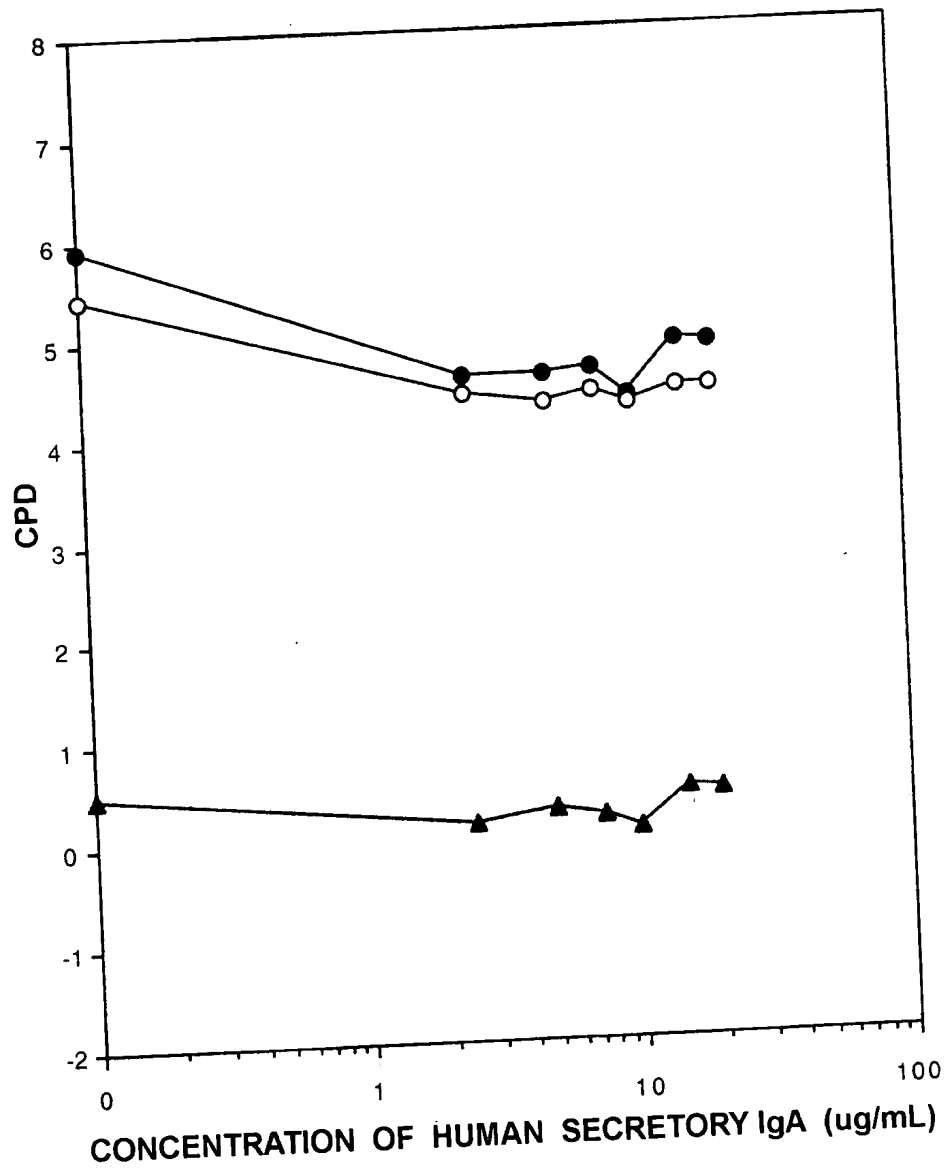
LEGEND:

Closed circles = + E$_2$

Open circles = − E$_2$

Closed triangles = Estrogenic effect

FIGURE 90

EFFECT OF MOUSE IgA ON H301 CELL
GROWTH IN SERUM-FREE MEDIUM

LEGEND:

Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 91

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON H301CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND: Clos d circles = + E_2

Open circles = $-E_2$

Closed triangles = Estrogenic effect

FIGURE 92

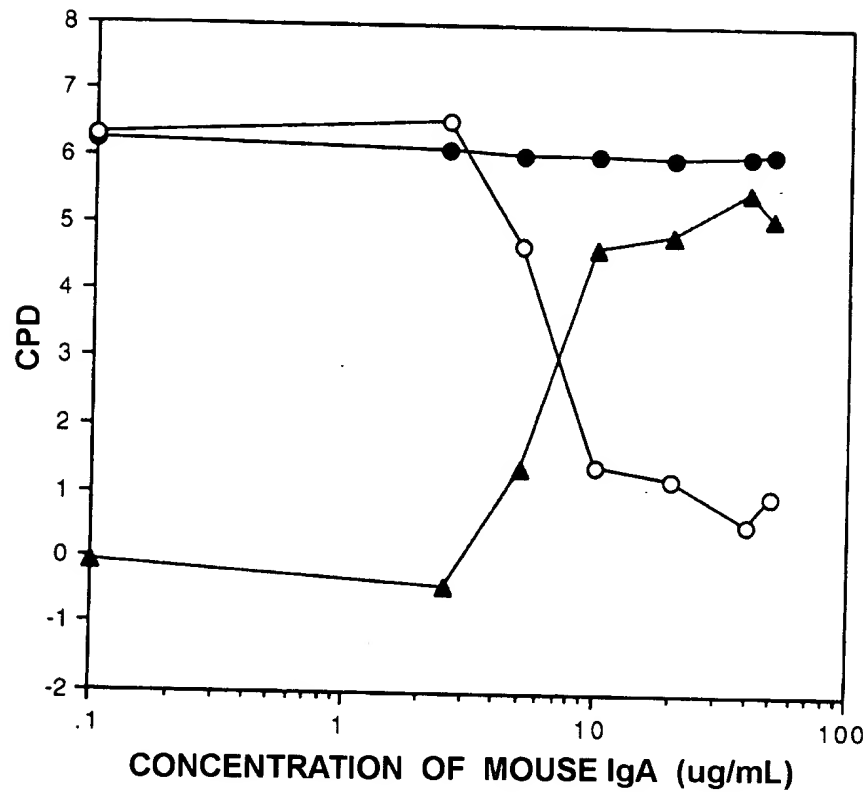## EFFECT OF ESTRADIOL ON H301 CELL GROWTH IN SERUM-FREE MEDIUM AND 40 ug/mL OF HUMAN IgM

ESTRADIOL CONCENTRATION (M)

FIGURE 93

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM
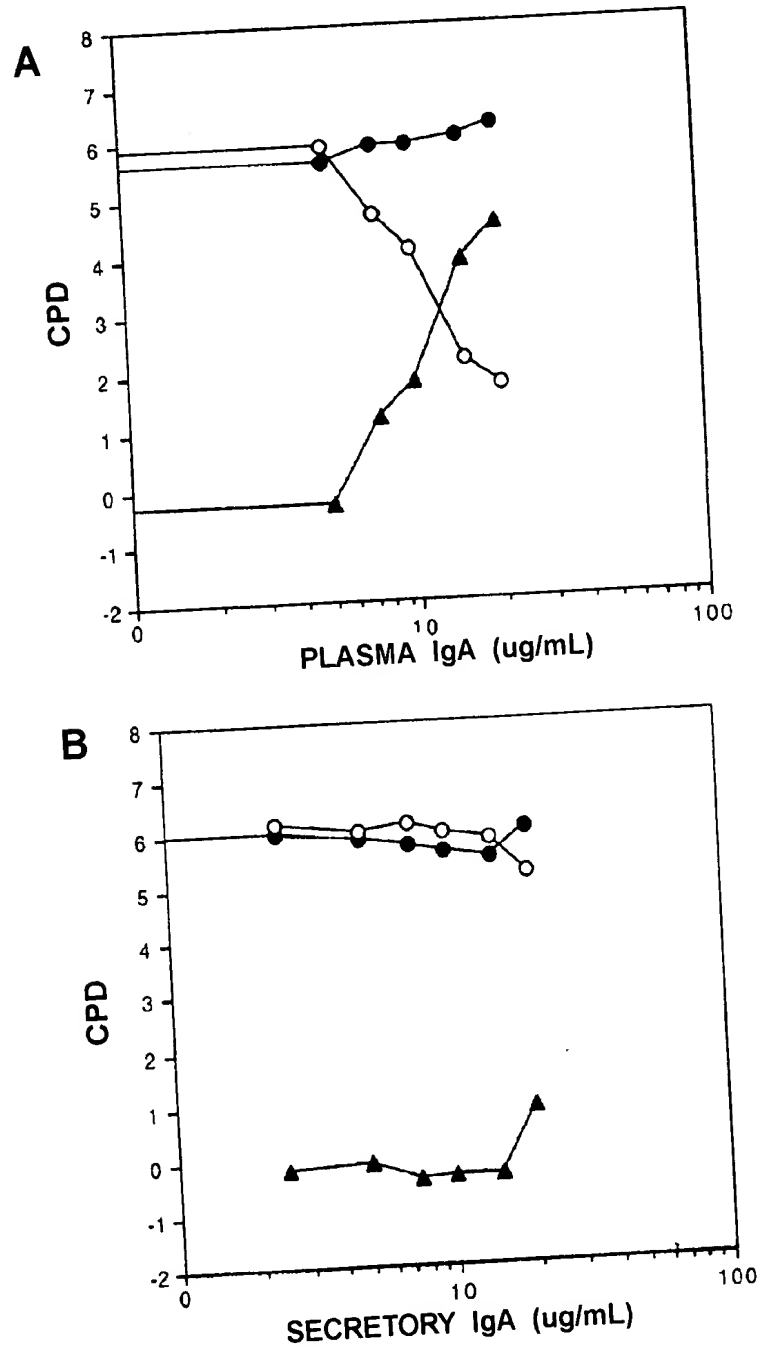

LEGEND: Closed circles = + E$_2$
Open circles = − E$_2$
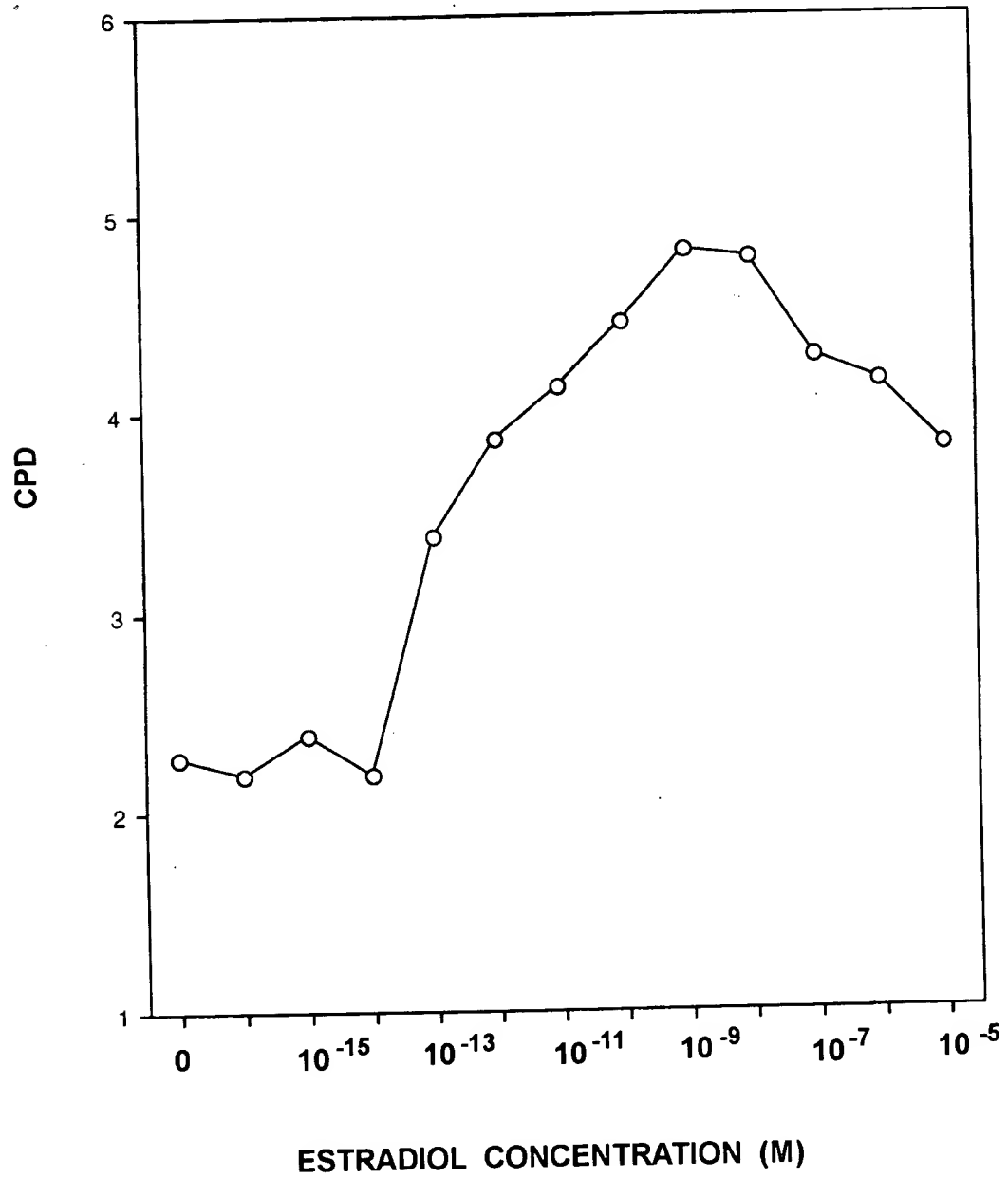Closed triangles = Estrogenic effect

FIGURE 94

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND: Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 95

EFFECT OF HUMAN IgM ON MCF-7A CELL
GROWTH IN SERUM-FREE MEDIUM

LEGEND:

—●— = + E_2

—○— = − E_2

—▼— = Estrogenic effect

FIGURE 96

EFFECT OF HUMAN IgM ON MCF-7K
CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND:

—●— = + E_2

—○— = − E_2

—▼— = Estrogenic effect

FIGURE 97

EFFECT OF ESTRADIOL ON MCF-7K CELL GROWTH
IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM

CONCENTRATION OF ESTRADIOL (M)
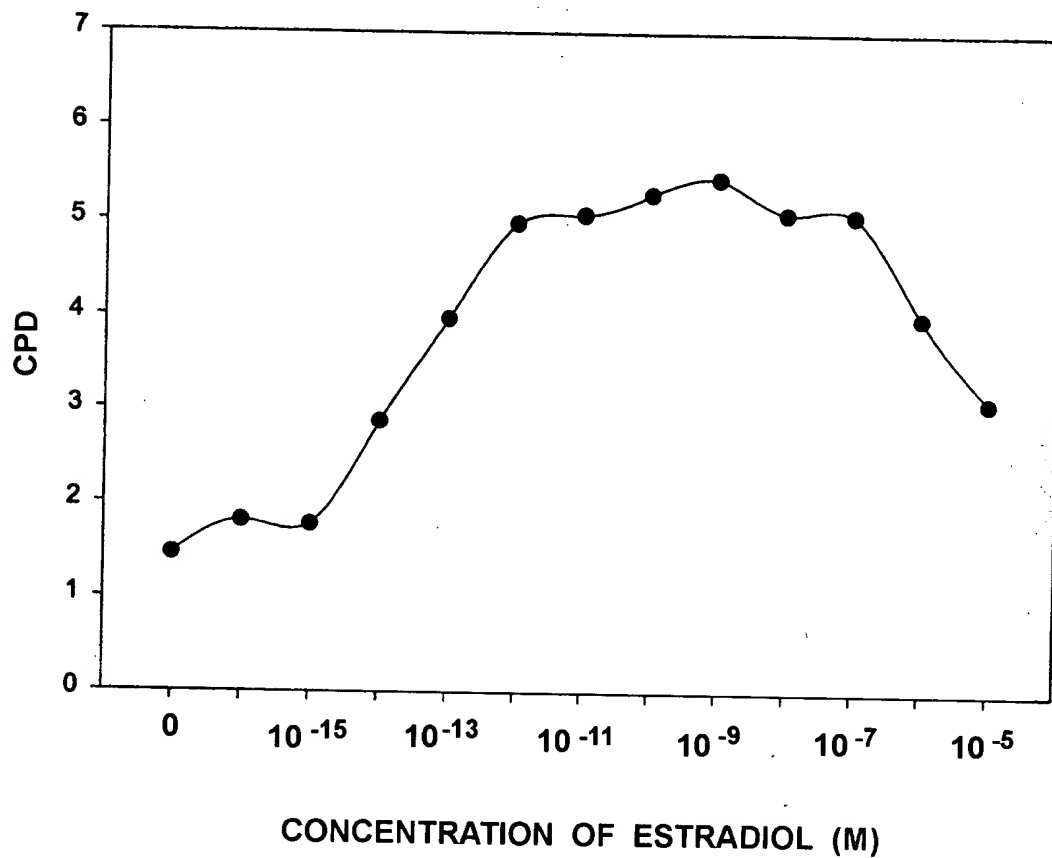
FIGURE 98

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON T47D CELL GROWTH IN SERUM-FREE MEDIUM
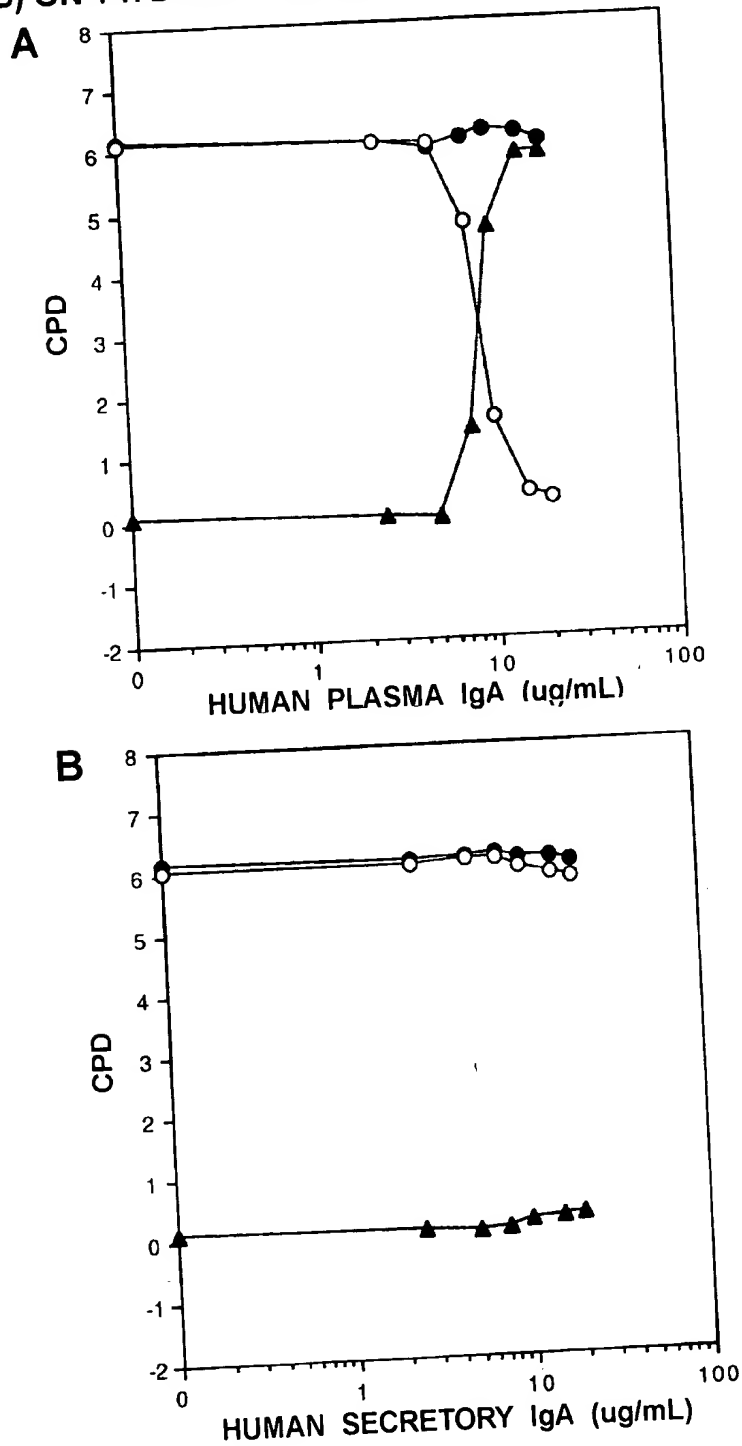
LEGEND: Closed circles = + E_2

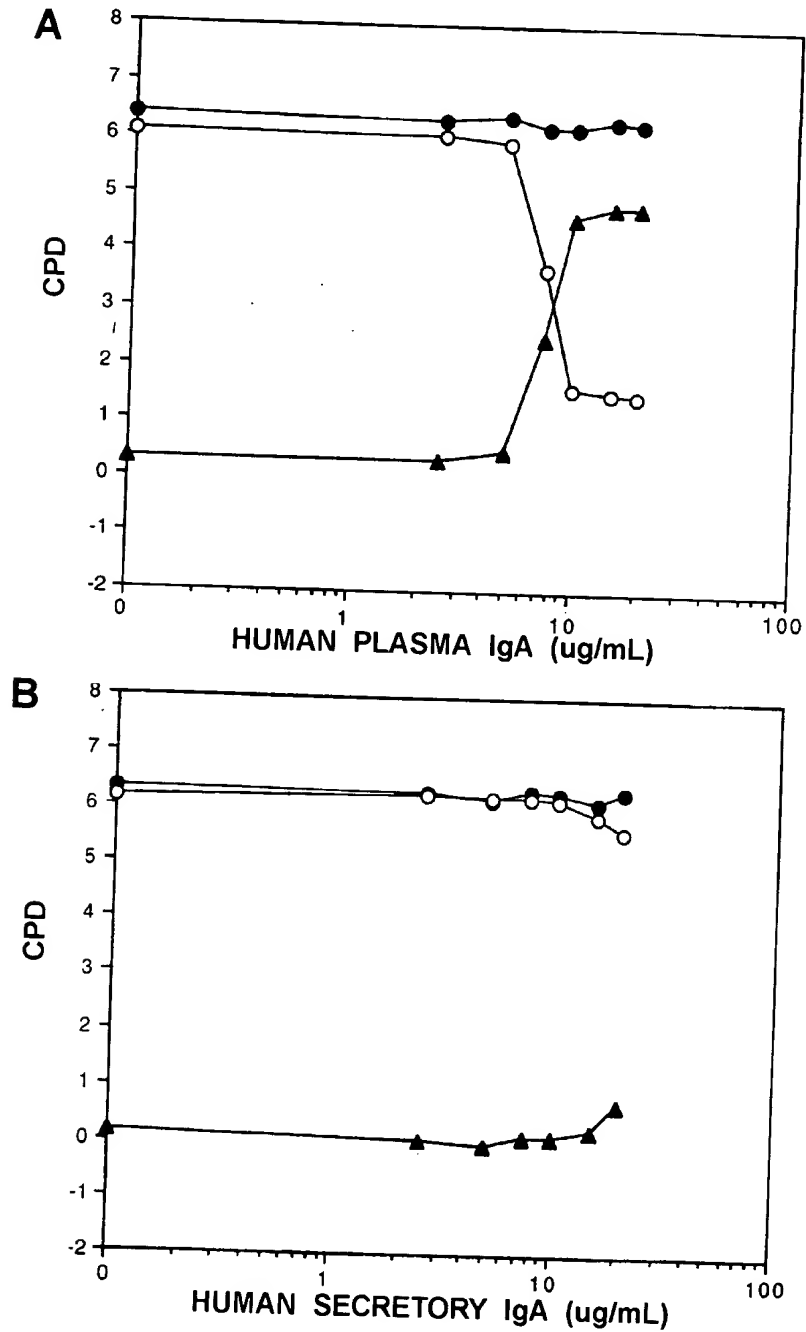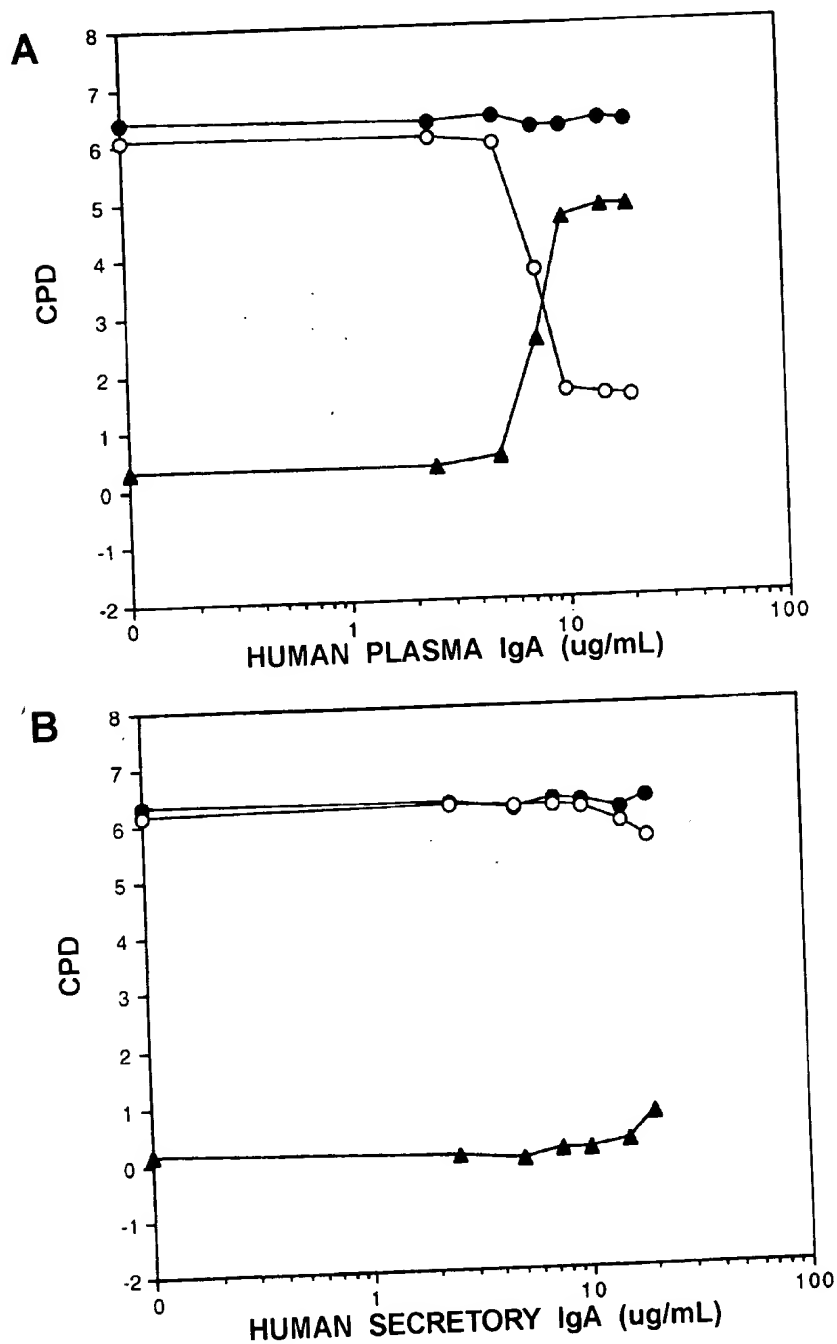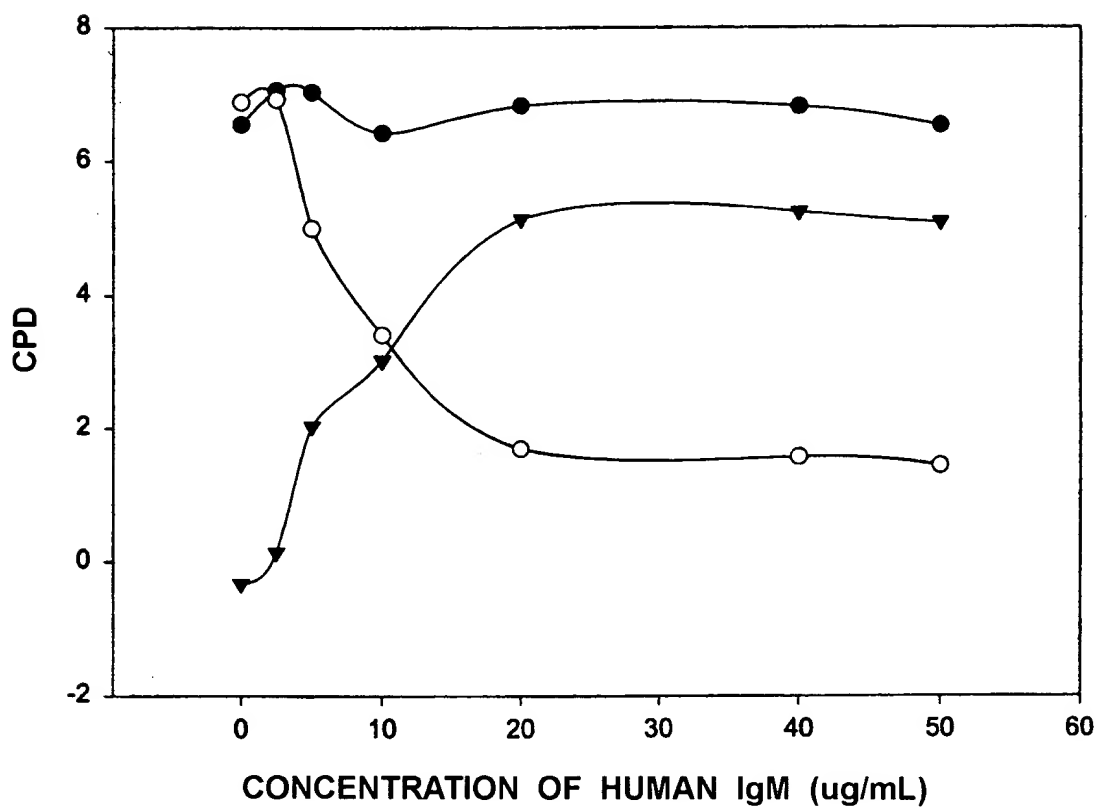Open circles = – E_2

Closed triangles = Estrogenic ffect

FIGURE 99

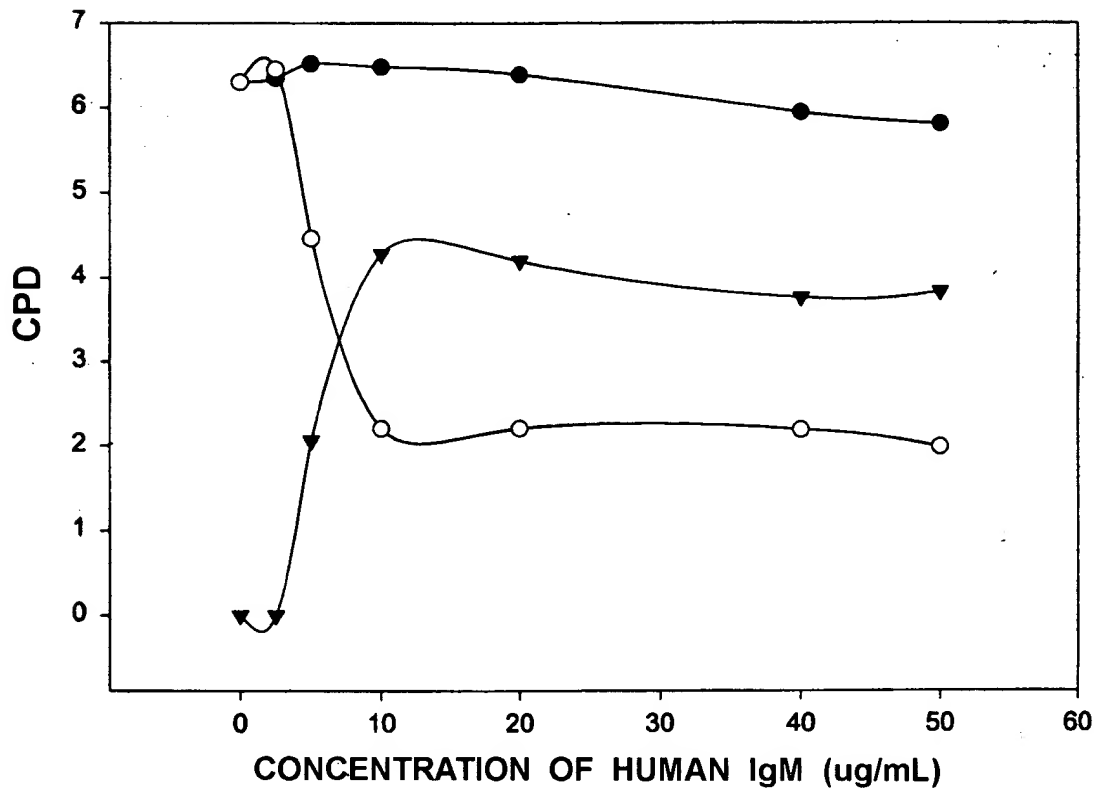## EFFECT OF HUMAN IgM ON T47D CELL GROWTH IN SERUM-FREE MEDIUM

LEGEND: Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 100

EFFECT OF ESTRADIOL ON T47D CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM

CONCENTRATION OF ESTRADIOL (M)

FIGURE 101

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM
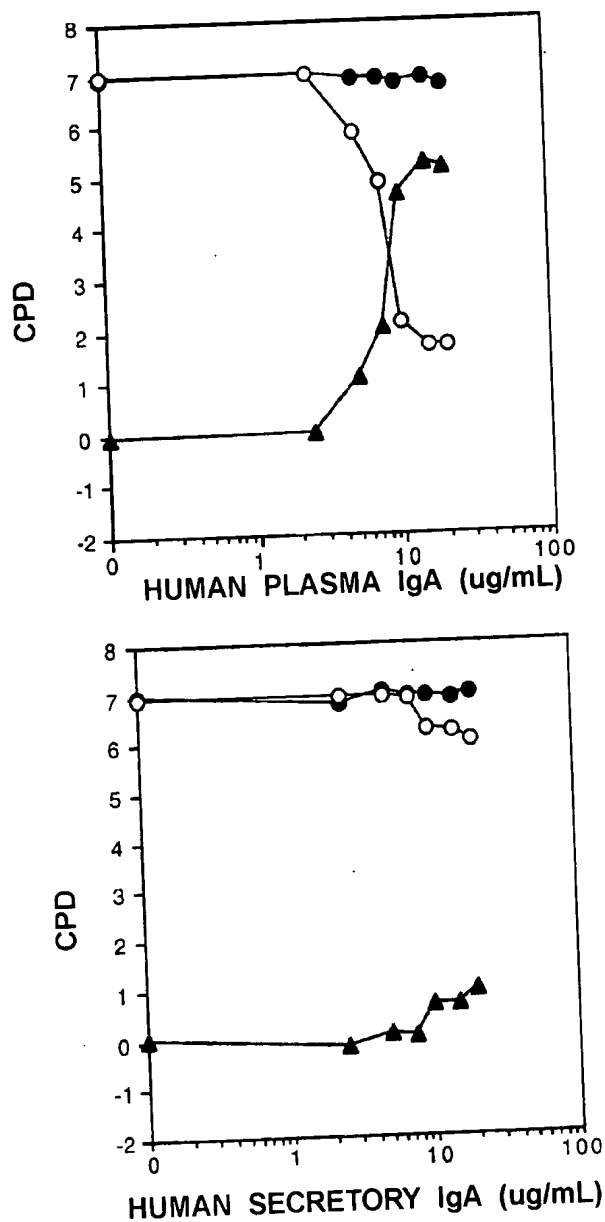
LEGEND: Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 102

EFFECT OF HUMAN PLASMA IgM ON
ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM

CONCENTRATION OF HUMAN PLASMA IgM (ug/mL)

LEGEND:

$-\bullet- = - E_2$

$-\circ- = + E_2$

FIGURE 103

EFFECT OF HUMAN IgM ON HT-29 CELL GROWTH IN THE PRESENCE OF INCREASING CONCENTRATIONS OF T_3
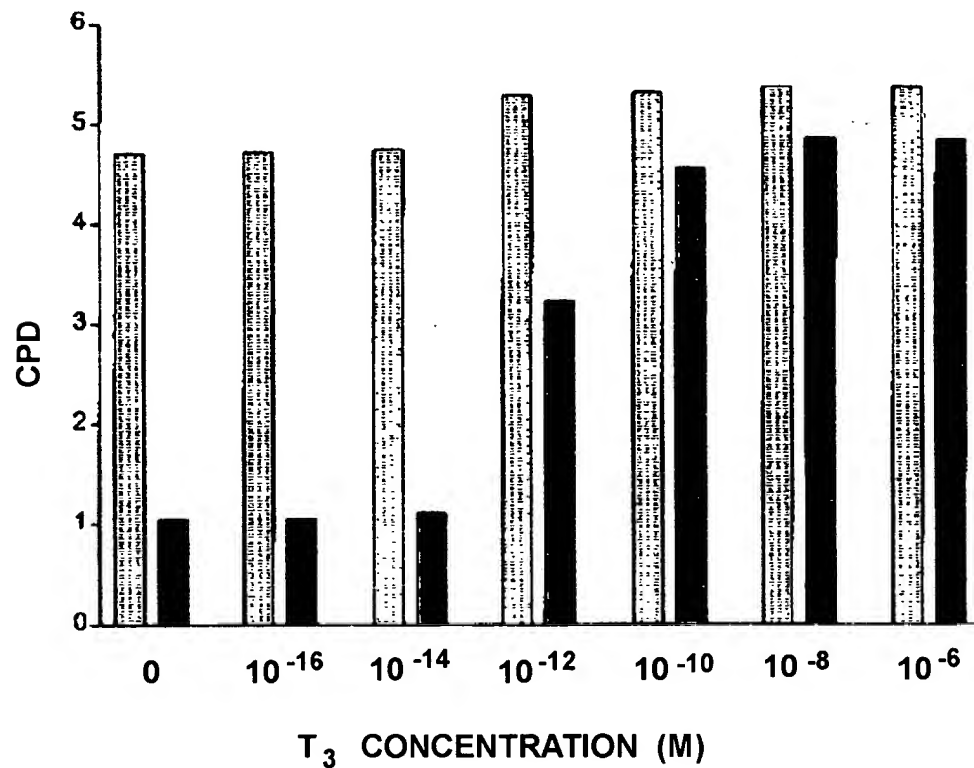
LEGEND:

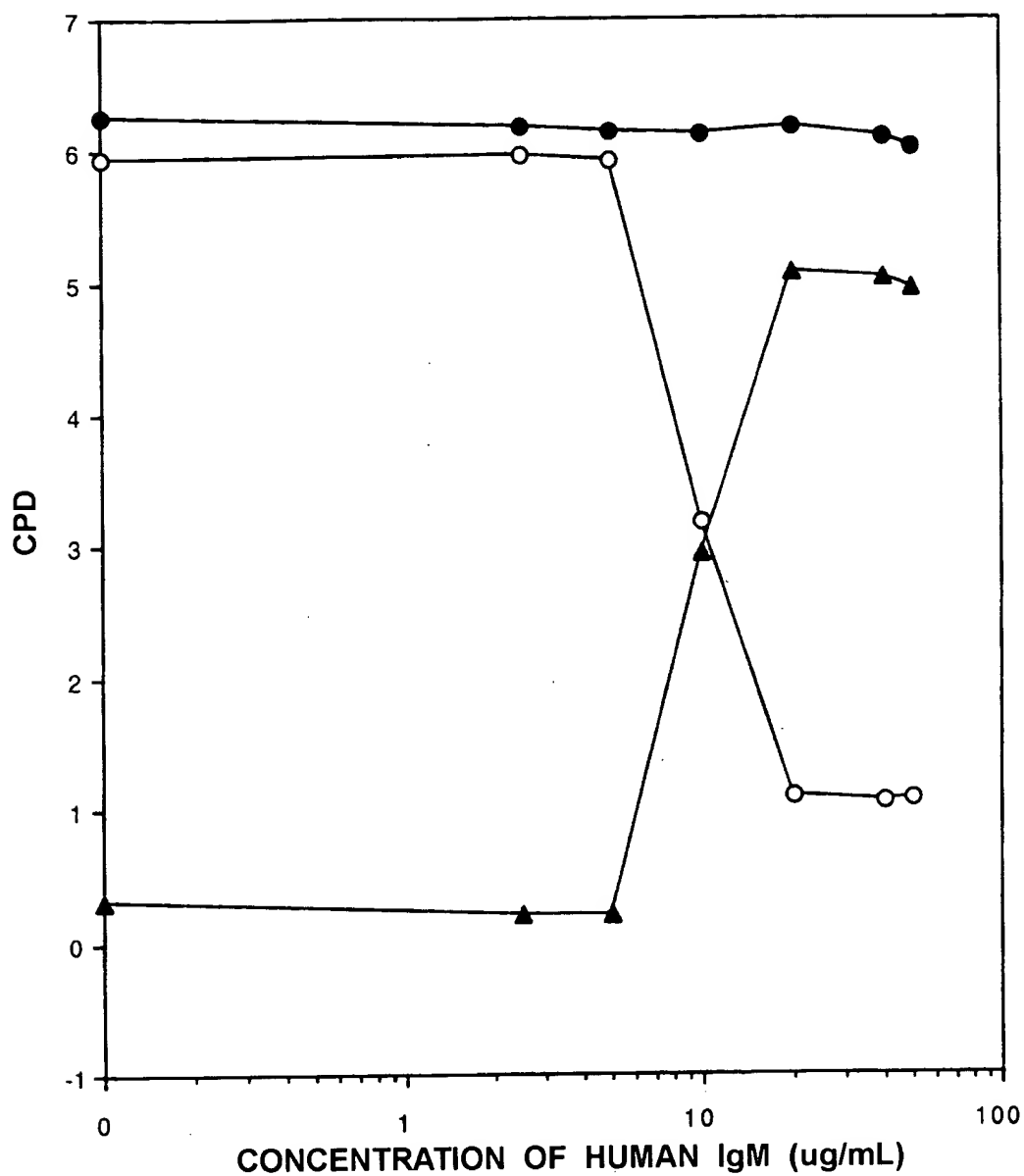▭ = T_3 Titration

▬ = T_3 Titration + 40 ug/mL IgM

FIGURE 104

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM
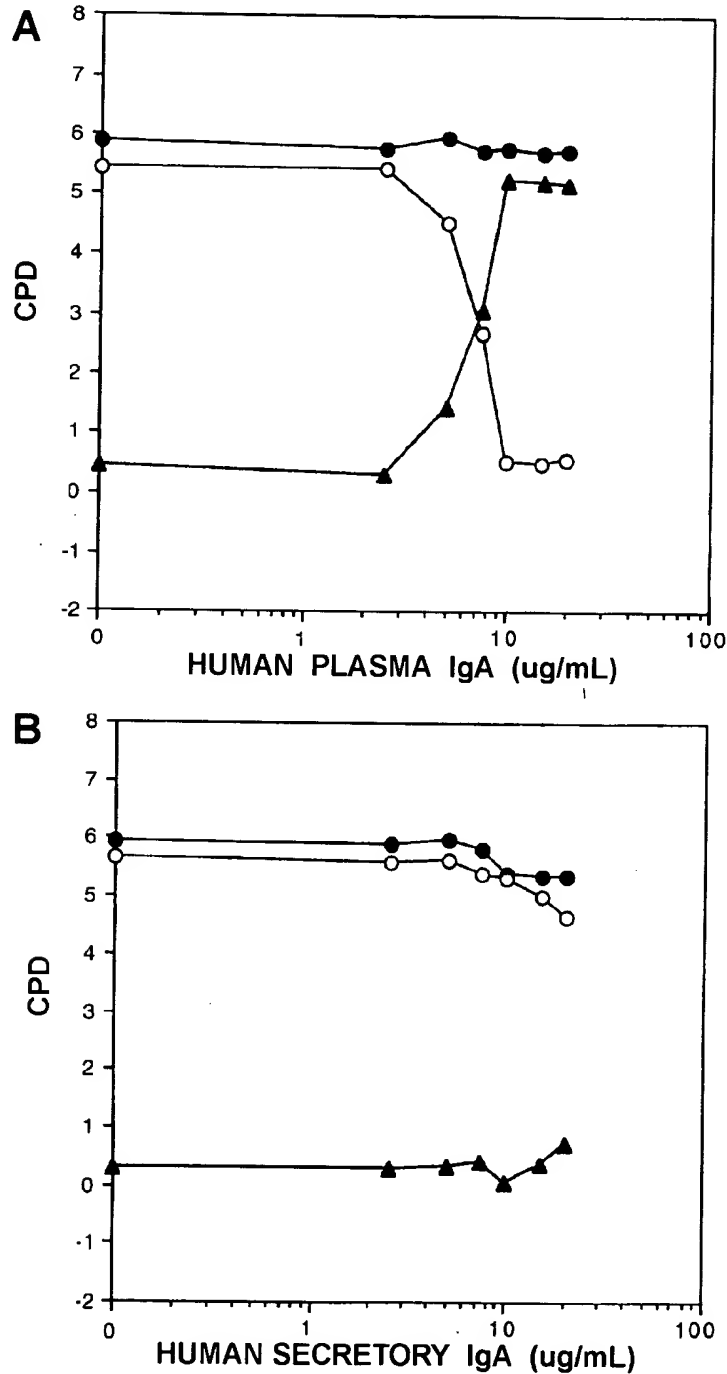
LEGEND: Closed circl s = + E$_2$
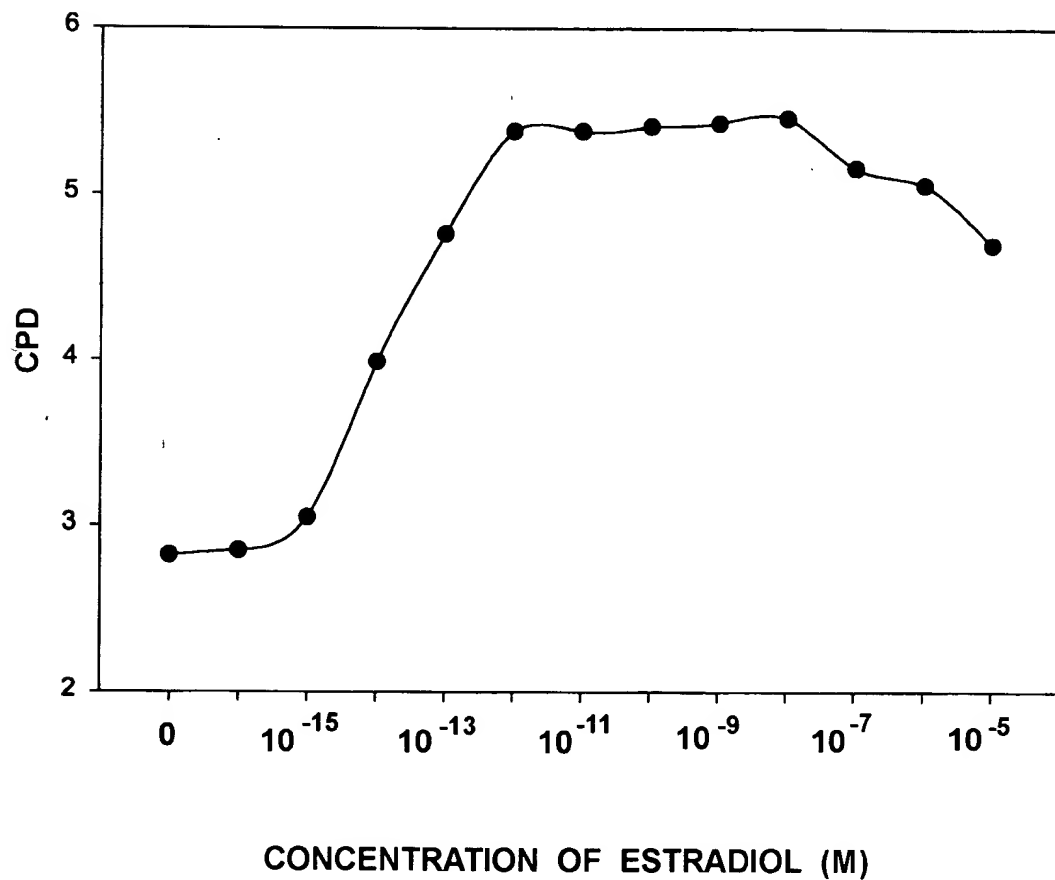Open circles = − E$_2$
Closed triangles = Estrogenic effect

FIGURE 105

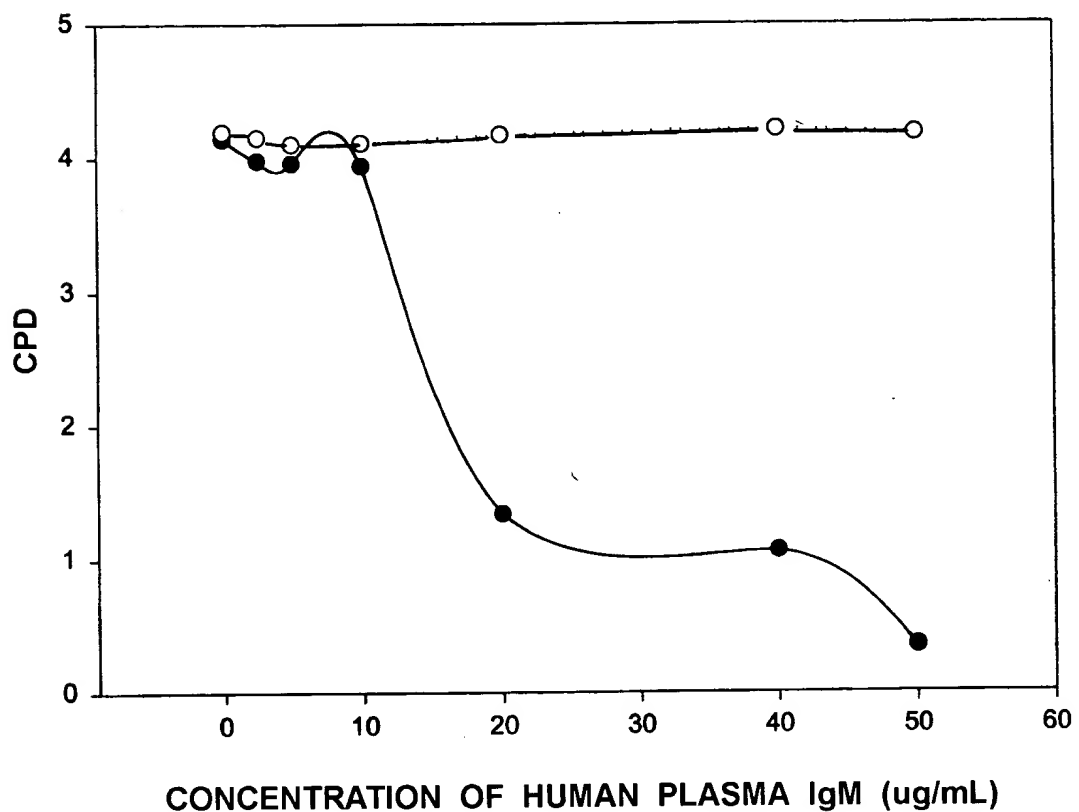## EFFECTS OF HUMAN PLASMA IgM VS IgM DERIVED FROM MYELOMA CELLS ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM WITH AND WITHOUT DHT
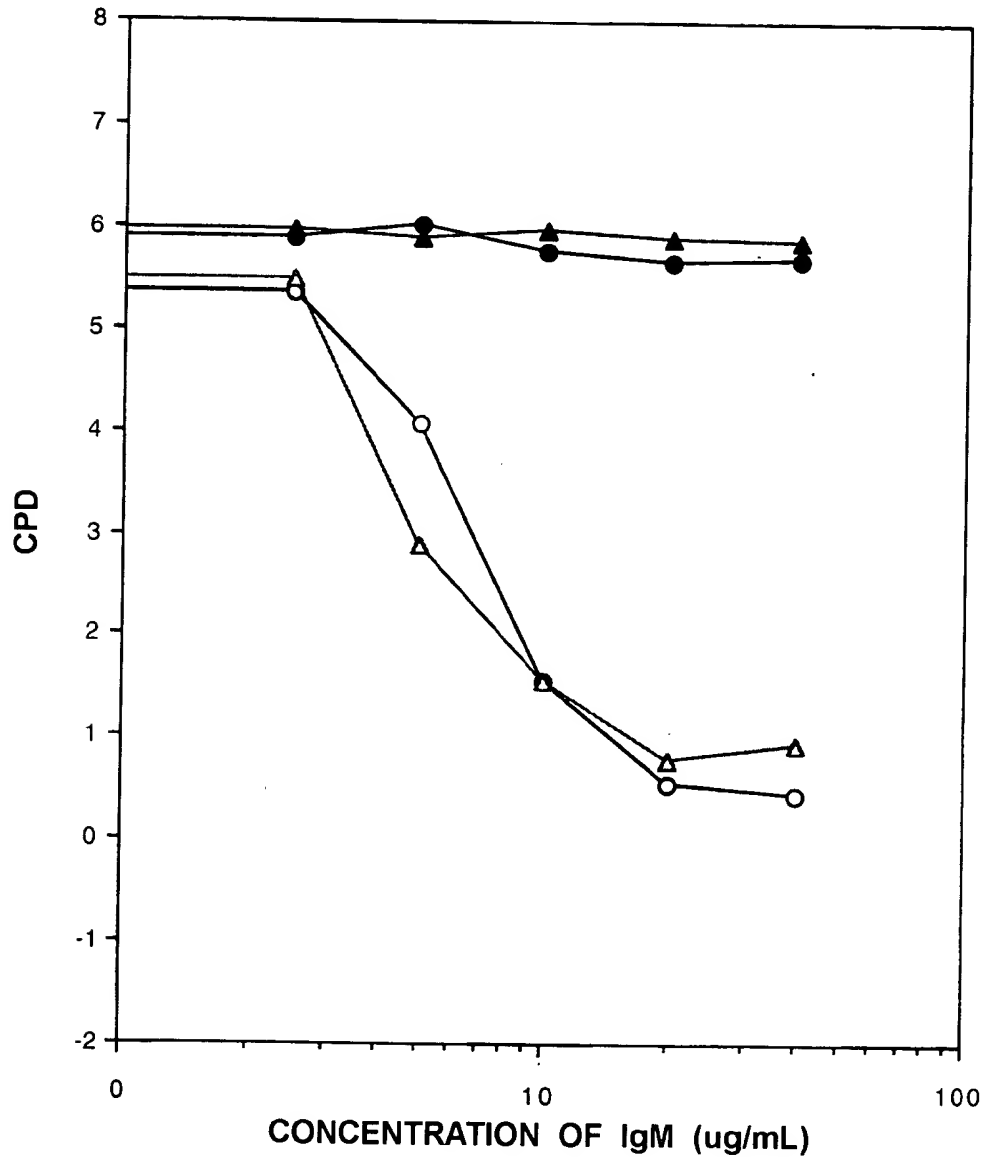
LEGEND:

—●— = DHT + Myeloma IgM

—○— = Myeloma IgM only

—▲— = DHT + Plasma IgM

—△— = Plasma IgM only

FIGURE 106

ESTROGENIC EFFECT OF 50 ug/mL OF VARIOUS IgM'S ON SEVERAL DIFFERENT CELL LINES

CELL LINE AND TYPE OF IgM
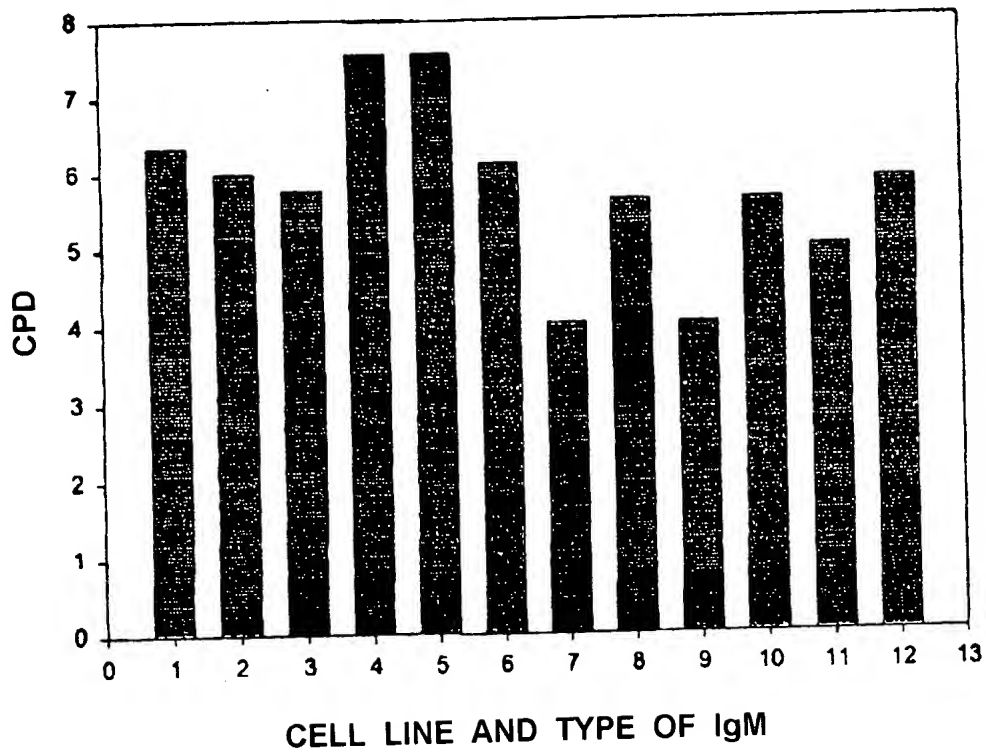
LEGEND:

1. Human IgM on MTW9/PL2 Cells = 6.36 cpd
2. Mouse IgM on MTW9/PL2 Cells = 6.00 cpd
3. Rat IgM on MTW9/PL2 Cells = 5.77 cpd
4. Human IgM on H301 Cells = 7.57 cpd
5. Mouse IgM on H301 Cells = 7.56 cpd
6. Rat IgM on H301 Cells = 6.11 cpd
7. Human IgM on GH1 Cells = 4.12 cpd
8. Rat IgM on GH1 Cells = 5.83 cpd
9. Human IgM on GH3 Cells = 4.09 cpd
10. Human IgM on GH4 Cells = 5.41 cpd
11. Human IgM on MCF-7A Cells = 5.01 cpd
12. Human IgM on MCF-7K C lls = 5.89 cpd

FIGURE 107

EFFECT OF TAMOXIFEN ON T47D CELL GROWTH IN DDM-2MF DEFINED MEDIUM
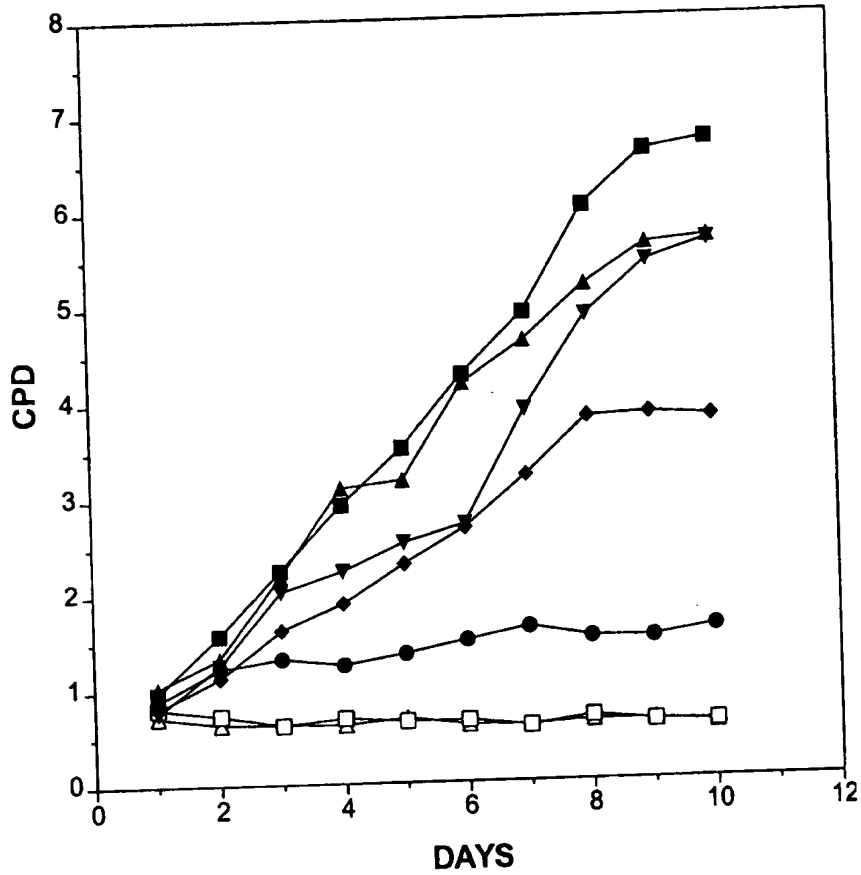
LEGEND:
- ■ SFM + E_2
- ▲ SFM - E_2
- ▼ SFM + 10^{-9} M TAM
- ♦ SFM + 10^{-8} M TAM
- ● SFM + 10^{-7} M TAM
- □ SFM + 10^{-6} M TAM
- △ SFM + 10^{-5} M TAM

FIGURE 108

EFFECT OF INCREASING ESTRADIOL CONCENTRATIONS ON T47D CELL GROWTH IN SERUM-FREE AND PHENOL- RED FREE MEDIUM WITH 10^{-7} TAMOXIFEN
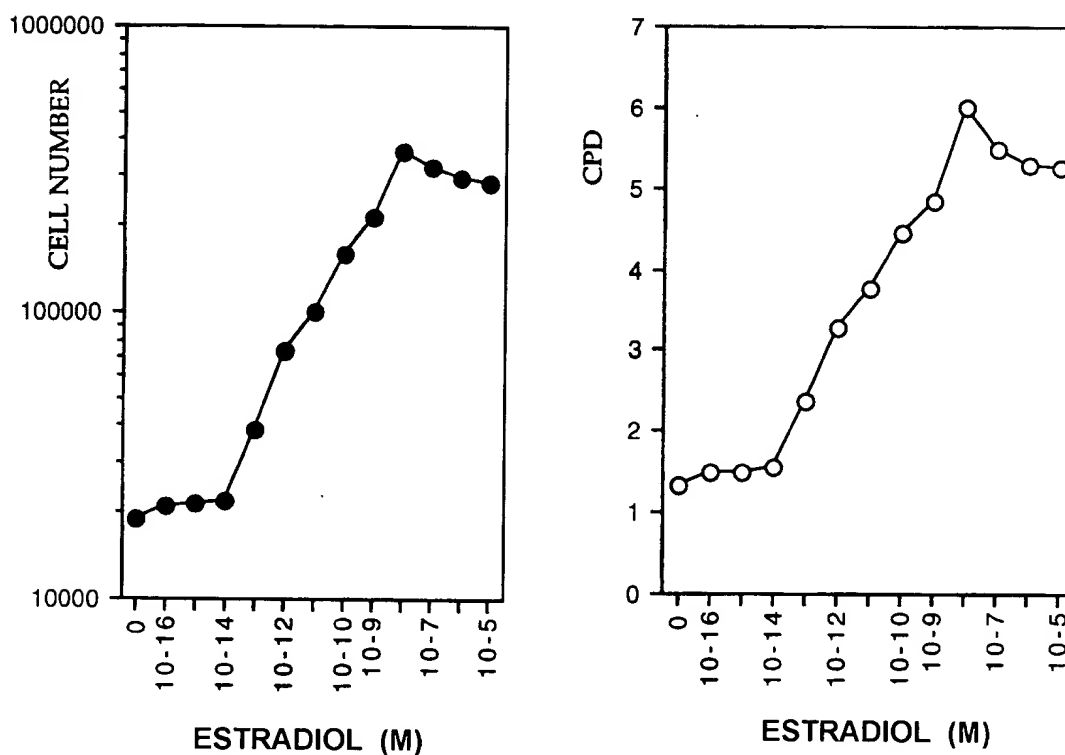
NOTE:

DATA ARE EXPRESSED AS BOTH CELL NUMBER AND CPD

FIGURE 109

E$_2$ RESCUE OF MTW9/PL2 CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HORSE IgM
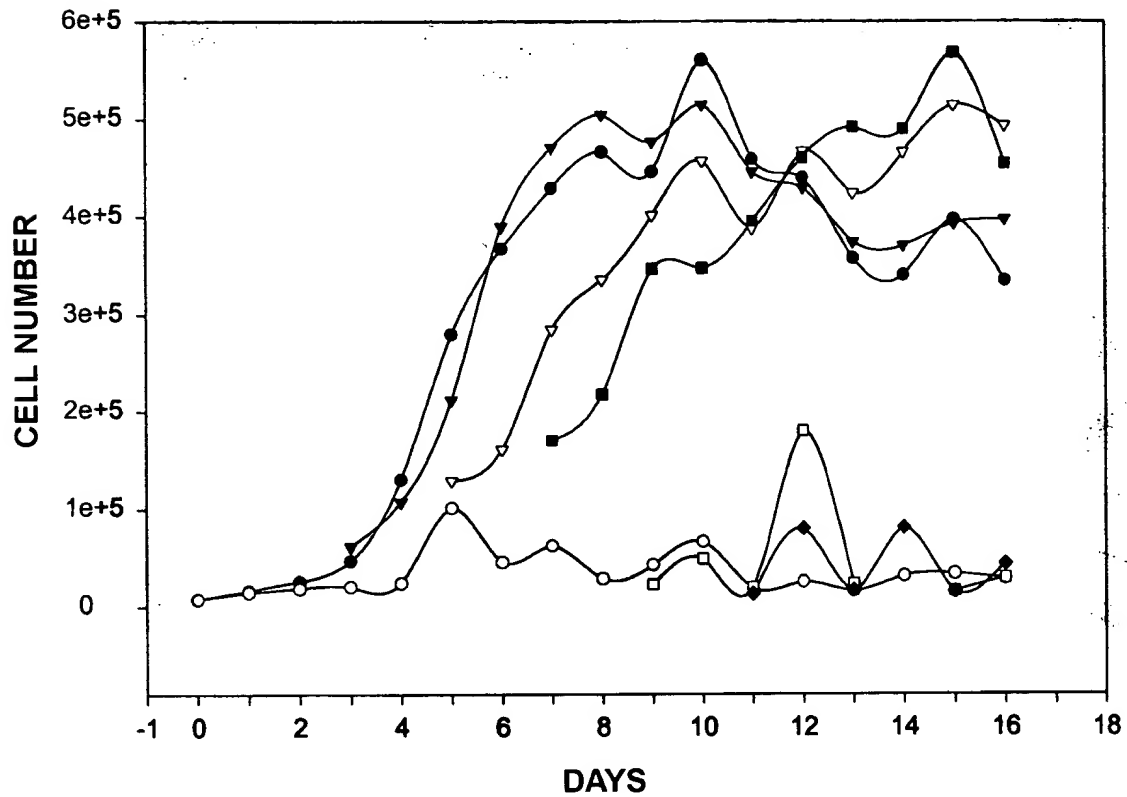
LEGEND:

—●— = E$_2$ Added on Day 0

—○— = No E$_2$

—▼— = E$_2$ Added on Day 2

—▽— = E$_2$ Added on Day 4

—■— = E$_2$ Added on Day 6

—□— = E$_2$ Added on Day 8

—◆— = E$_2$ Added on Day 10

FIGURE 110

SUMMARY OF E_2 RESCUE OF MTW9/PL2 CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HORSE IgM
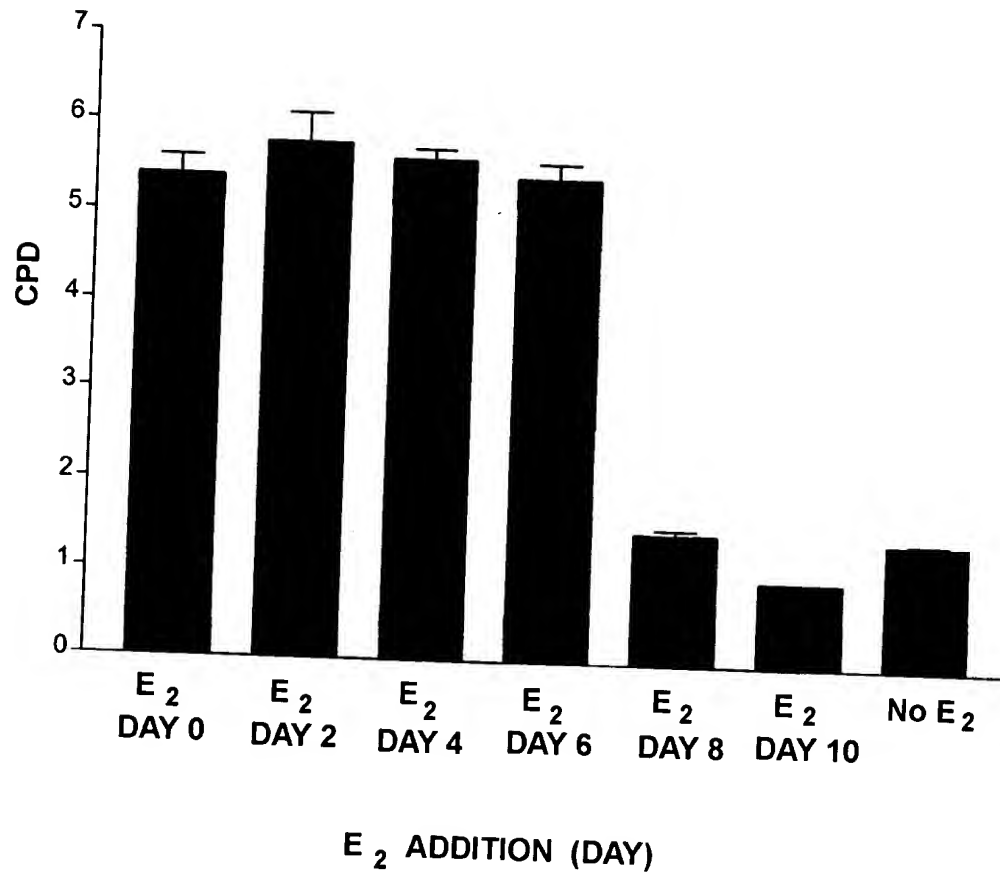
FIGURE 111

E_2 RESCUE OF T47D CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HORSE IgM

E$_2$ ADDITION (DAY)
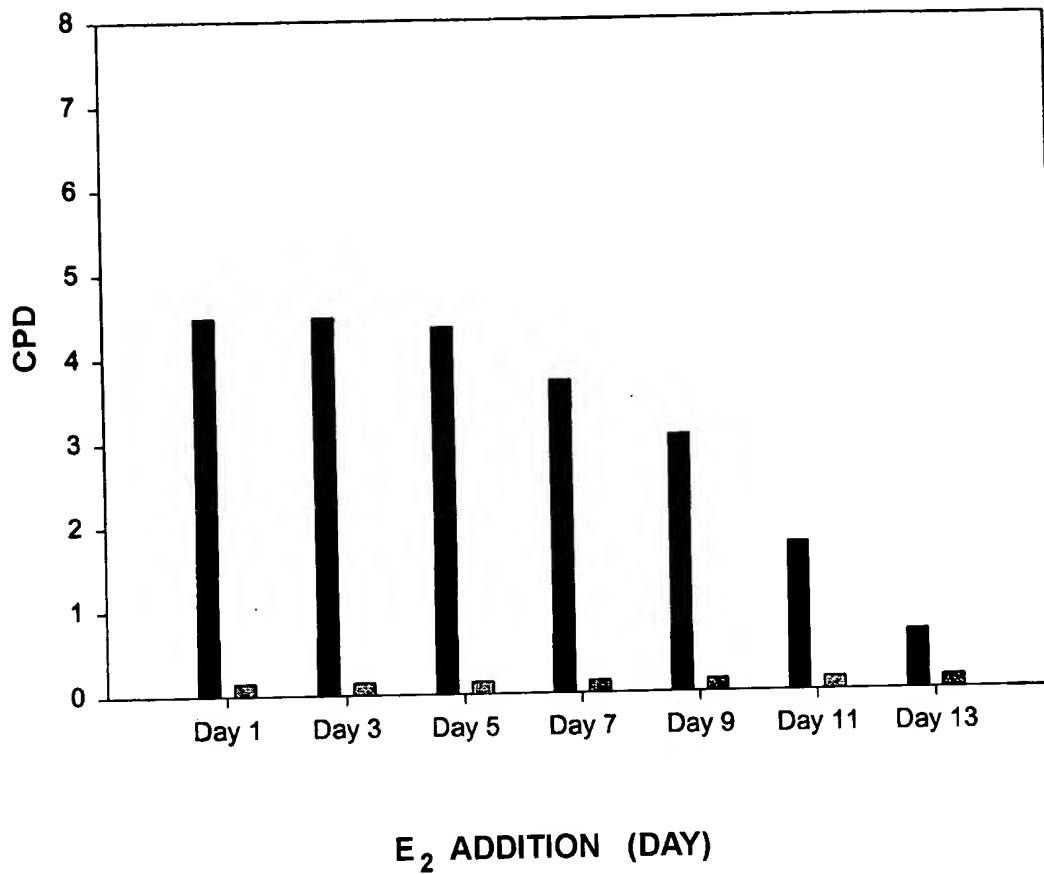
LEGEND:

███ = + E$_2$

▓▓▓ = − E$_2$

FIGURE 112

ESTROGEN RESCUE OF MCF-7A CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL OF HUMAN SERUM IgM

E_2 ADDITION (DAY)
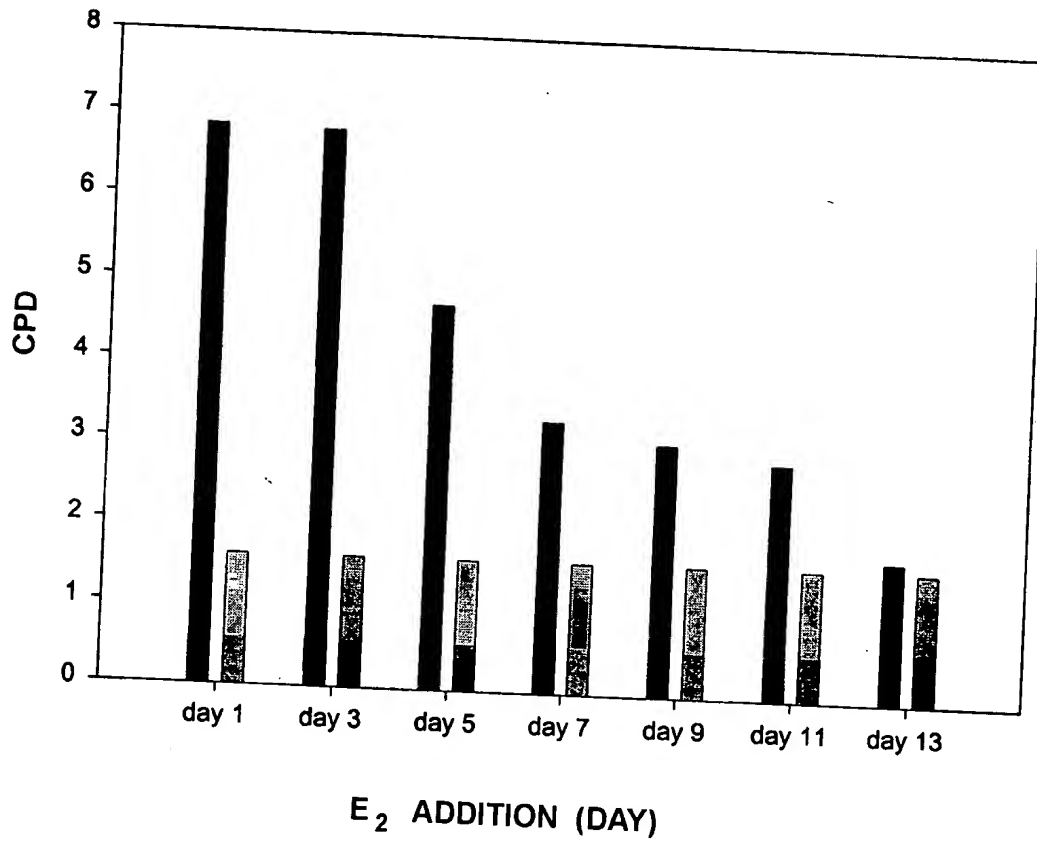
LEGEND:

■ = + E_2

▨ = − E_2

FIGURE 113

DETECTION OF SECRETORY COMPONENT
IN SECRETORY IgA WITH ANTI-SC ANTIBODY

MW

80 kDa

54 kDa

IgA   IgG   Secretory   Albumin
                    IgA
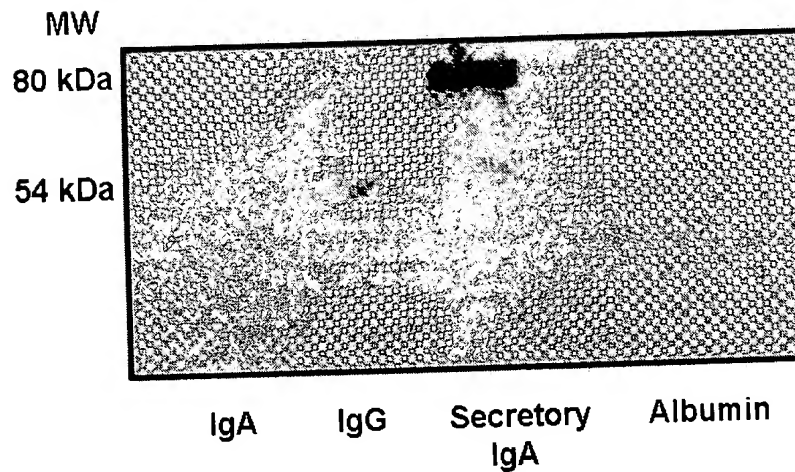
20 ug/mL APPLIED IN ALL LINES

IgA = Human Plasma

IgG = Human Plasma

Secretory IgA = IgA from Milk

Albumin = Human

FIGURE 114

HUMAN IgM TITRATION ON T47D CELLS GROWN IN SERUM-FREE MEDIUM WITH DIFFERENT DILUTIONS OF ANTI-SC ANTIBODY
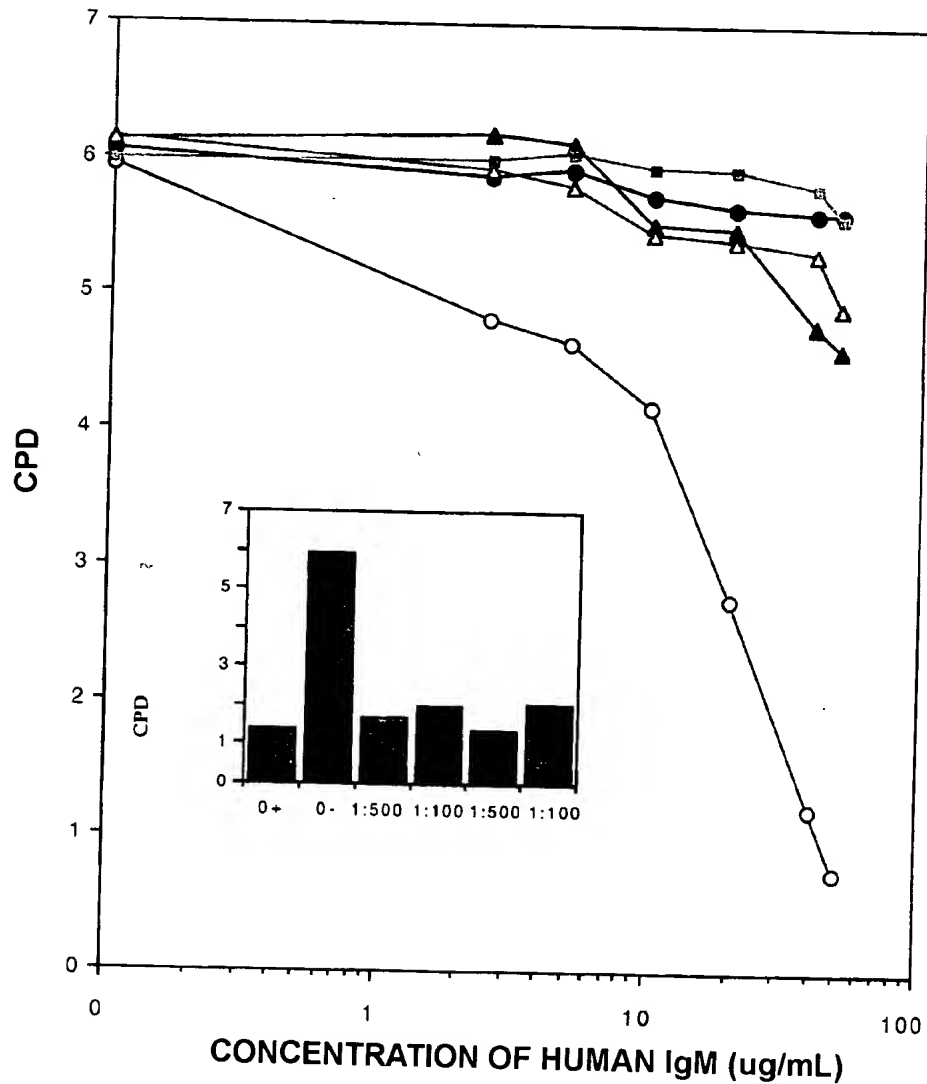
LEGEND: ●— = + E_2

○— = − E_2

▲— = 1:5000 Dilution of Anti-SC Antibody

△— = 1:1000 Dilution of Anti-SC Antibody

■— = 1:500 Dilution of Anti-SC Antibody

INSERT: EFFECT OF RABBIT SERUM ON T47D CELLS INCUBATED WITH 40 ug/mL HUMAN IgM

FIGURE 115

EFFECT OF IgA ON LNCaP GROWTH IN THE PRESENCE OF ANTI-SECRETORY COMPONENT ANTIBODY AT DIFFERENT DILUTIONS
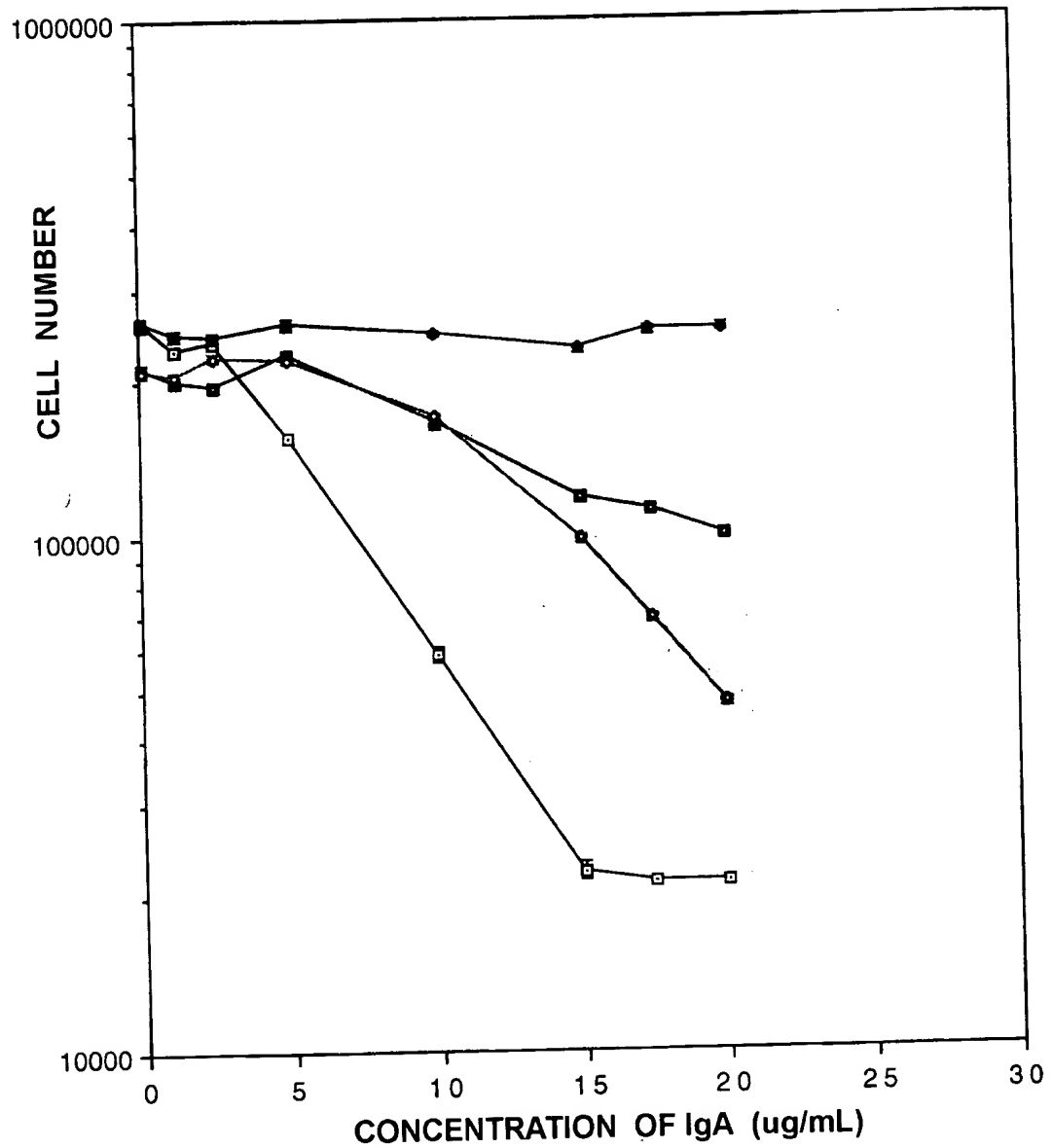
LEGEND: —□— = Control
—◆— = 1:100 Dilution of Anti-SC Antibody
—■— = 1:500 Dilution of Anti-SC Antibody
—◇— = 1:1000 Dilution of Anti-SC Antibody

FIGURE 116

WESTERN BLOT: ANTI-SECRETORY COMPONENT

MW

1   2   3   4   5   6   7   8   9   10   11   12
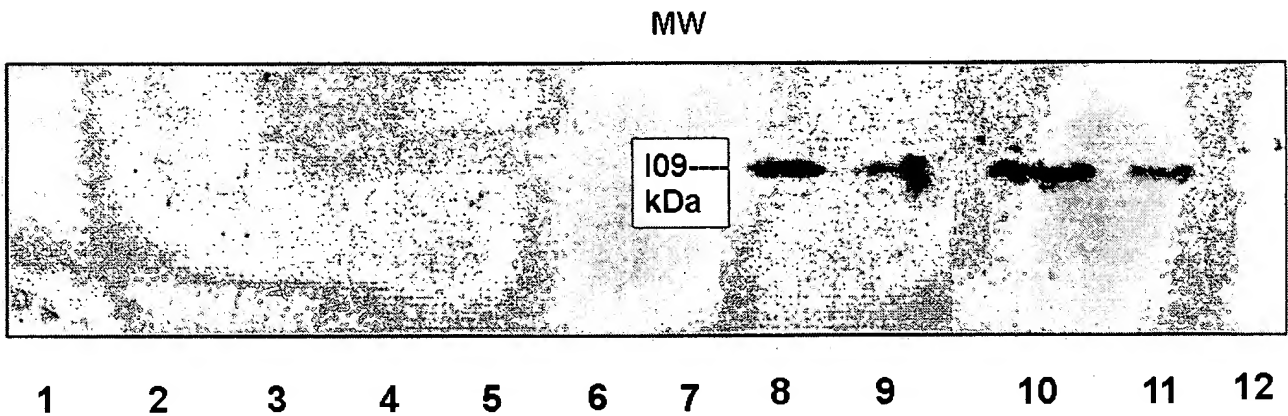
LEGEND:

1. MW
2. ALVA 41: 40 ug
3. ALVA 41: 20 ug
4. DU 145: 40 ug
5. DU 145: 20 ug
6. HUMAN FIBROBLAST: 40 ug
7. HUMAN FIBROBLAST: 20 ug
8. LNCaP: 40 ug
9. LNCaP: 20 ug
10. MDCK1: 20 ug
11. MDCK1: 10 ug
12. PC3: 40 ug

FIGURE 117

EFFECT OF HUMAN PLASMA IgA ON DU145
CELL GROWTH WITH AND WITHOUT DHT

CONCENTRATION OF IgA (ug/mL)
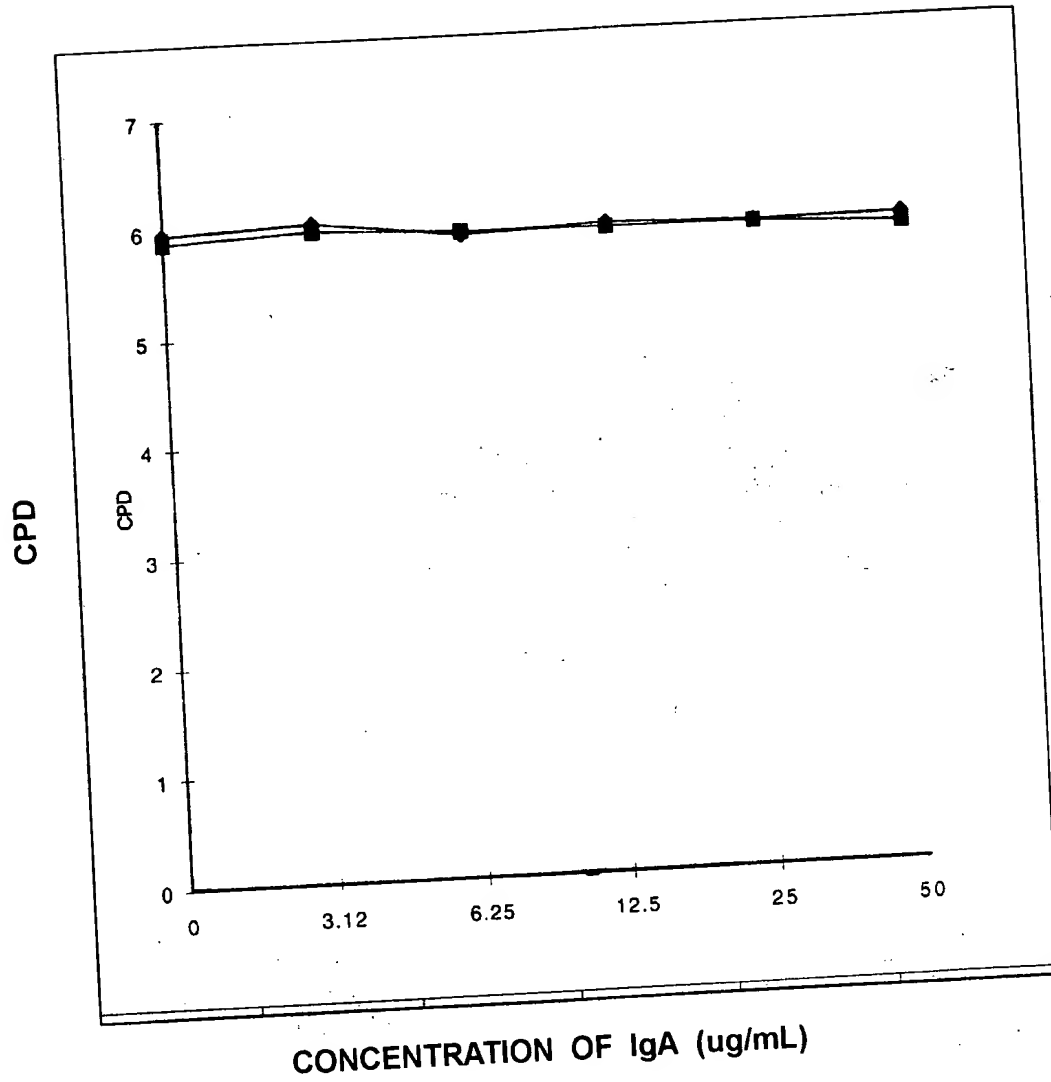
LEGEND:

◆ = + DHT

■ = - DHT

FIGURE 118

EFFECT OF HUMAN PLASMA IgA ON PC3 CELL GROWTH WITH AND WITHOUT DHT

CONCENTRATION OF IgA (ug/mL)
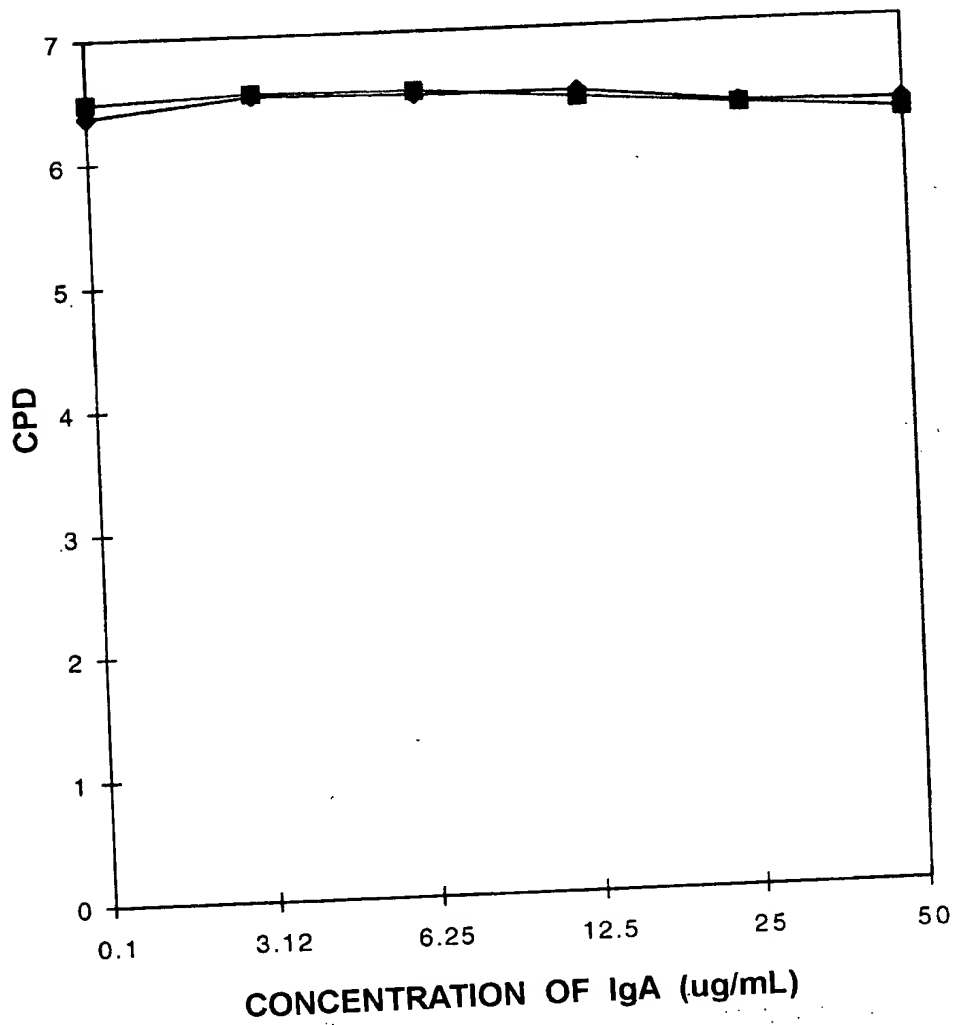
LEGEND:

◆ = + DHT

■ = − DHT

FIGURE 119

EFFECT OF RAT IMMUNOGLOBULINS ON MTW9/PL2
CELL GROWTH IN SERUM-FREE MEDIUM

CONCENTRATION OF RAT IMMUNOGLOBULINS (15 ug/mL)

LEGEND:

■ = + E_2

▨ = − E_2
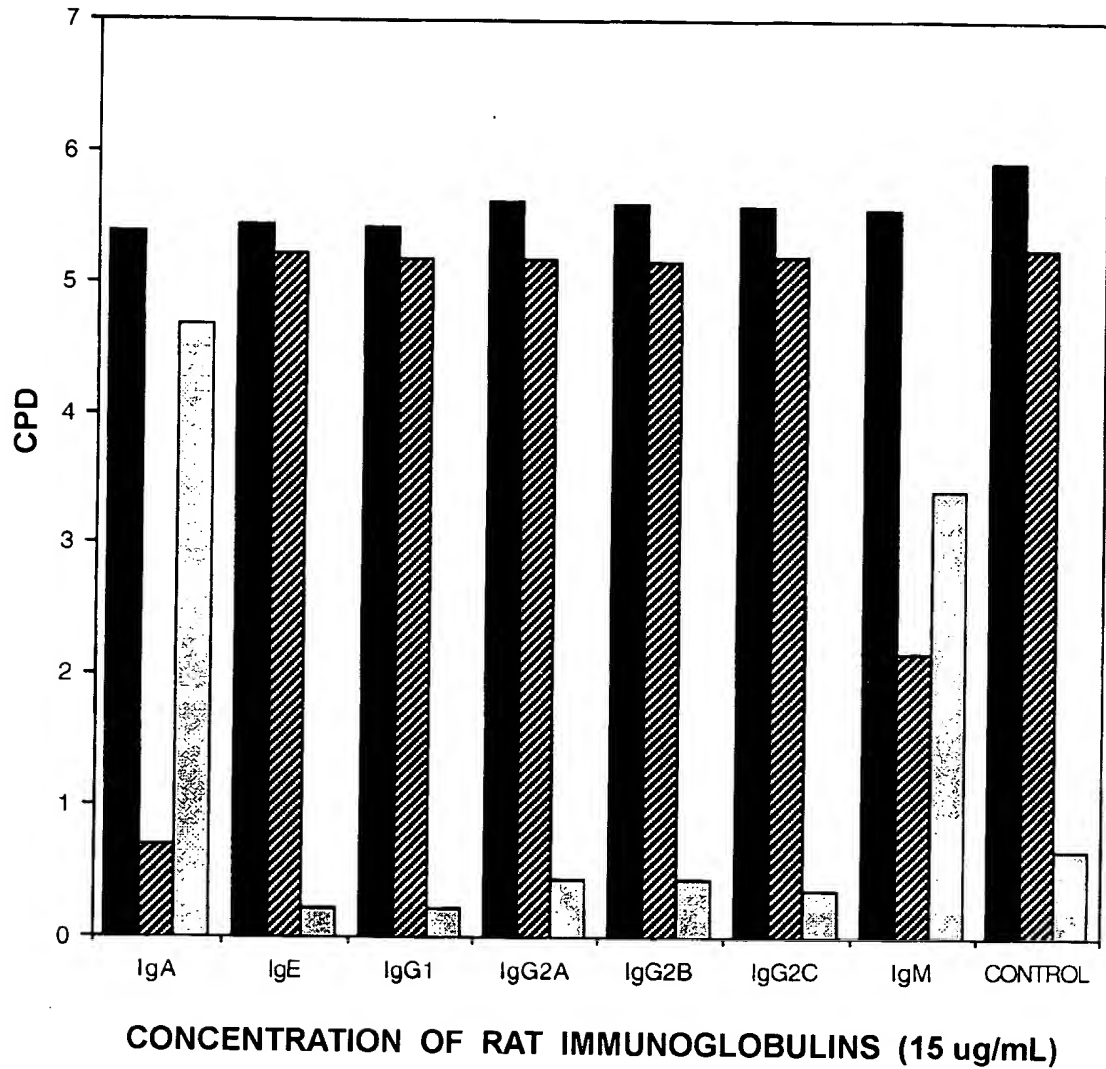
▣ = Estrogenic effect

CONTROL IS SERUM-FREE MEDIUM ALONE ± E_2

FIGURE 120

ESTROGENIC EFFECT GENERATED BY IMMUNOGLOBULINS

WITH T47D CELLS IN SERUM-FREE MEDIUM

IMMUNOGLOBULIN ADDED
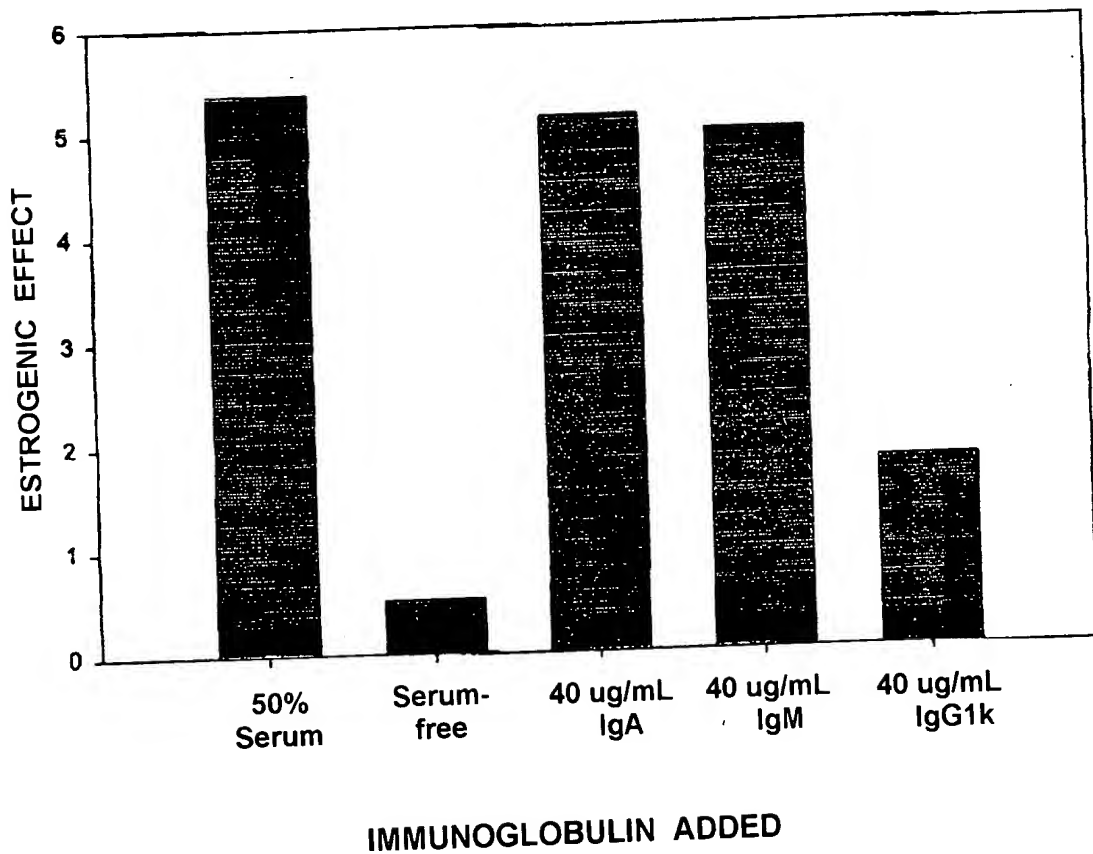
FIGURE 121

EFFECT OF IgG ISOTYPES (40 ug/mL) ON LNCaP
CELL GROWTH IN SERUM-FREE MEDIUM
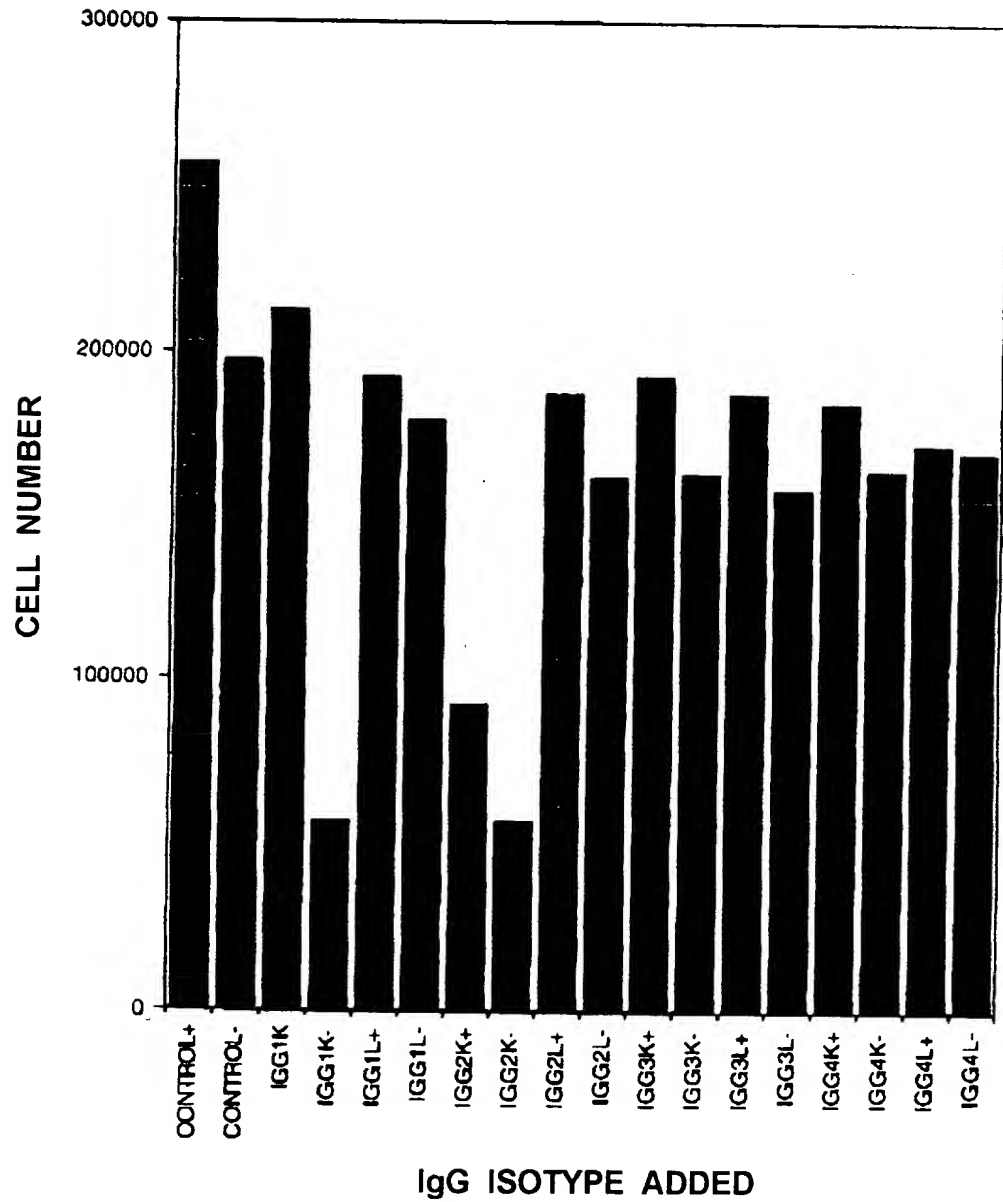
LEGEND: + = DHT Added
           − = No DHT Added

FIGURE 122

IgG ISOTYPE ASSAYS WITH LNCaP CELLS IN SERUM-FREE DEFINED MEDIUM ± DHT
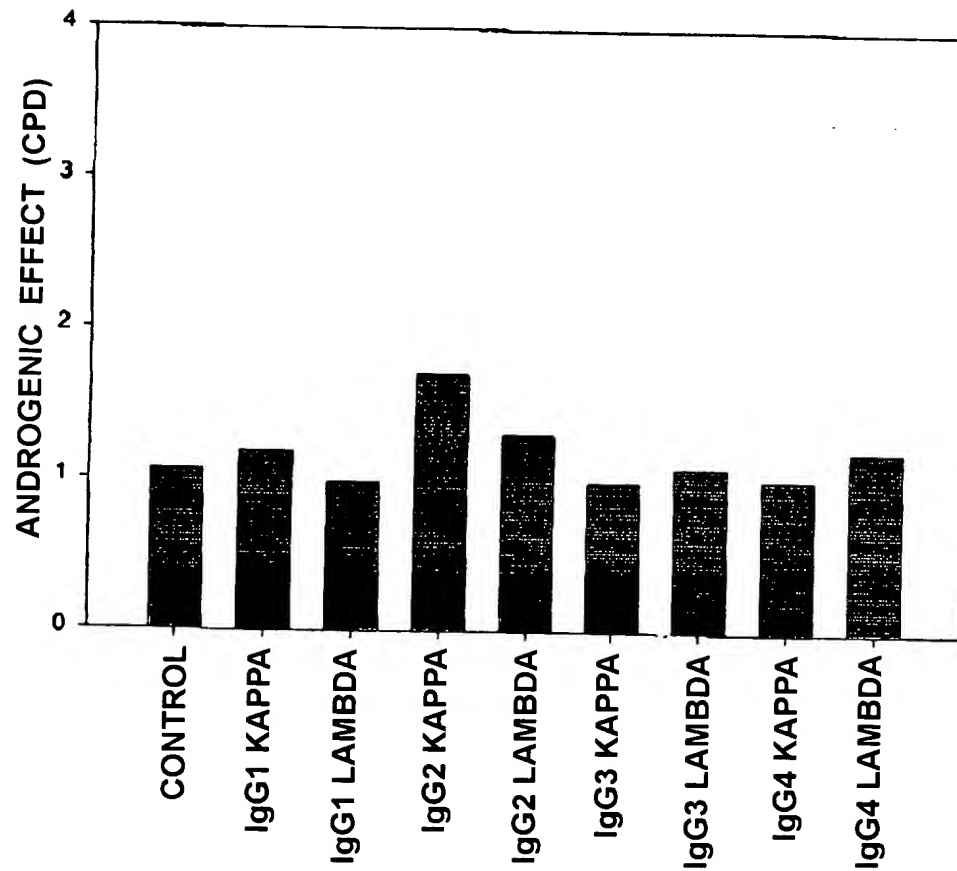
FIGURE 123

MODEL OF EARLY ONSET BREAST CANCER
INCLUDING TGF-BETA

ER$^+$ BREAST CANCERS

(i) Inhibitory receptor(s) for IgA & IgM & IgG1

(ii) Growth inhibition by IgA & IgM

(iii) Little or no TGFβ growth inhibition

(iv) No TGFβ receptors

↗ ↗ ↗

NORMAL EPITHELIAL CELLS

I. Inhibitory receptor(s) for IgA & IgM & IgG1 & TGFβ

II. Growth inhibition by IgA & IgM & TGFβ

↘ ↘ ↘

ER$^-$ BREAST CANCERS

(i) No functional receptors for IgA or IgM & IgG1

(ii) No growth inhibition by IgA & IgM

(iii) High sensitivity TGFβ growth inhibition

(iv) TGFβ receptors present

FIGURE 124

EFFECT OF CARCINOGENS ON MAMMARY TUMOR INDUCTION IN RATS OF VARIOUS AGES
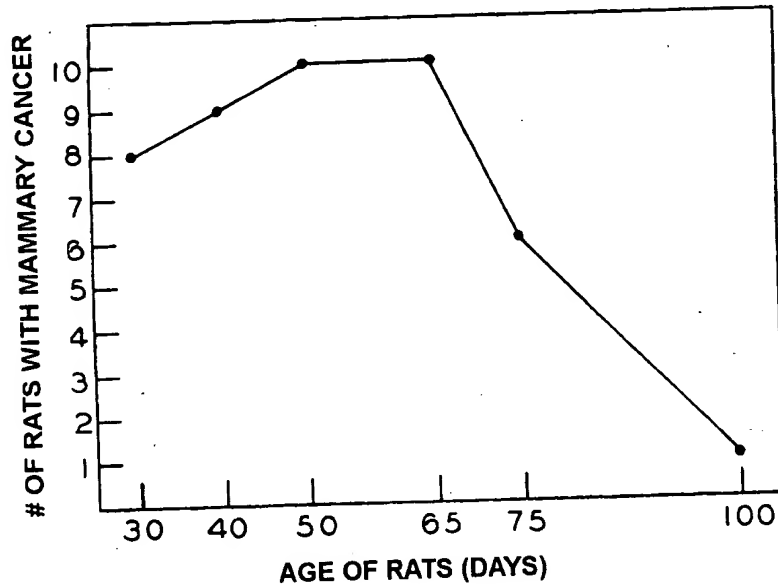
INCIDENCE OF MAMMARY CANCER IN GROUPS OF 10
FEMALE RATS OF VARIOUS AGES FED 3-MC, 100 MG
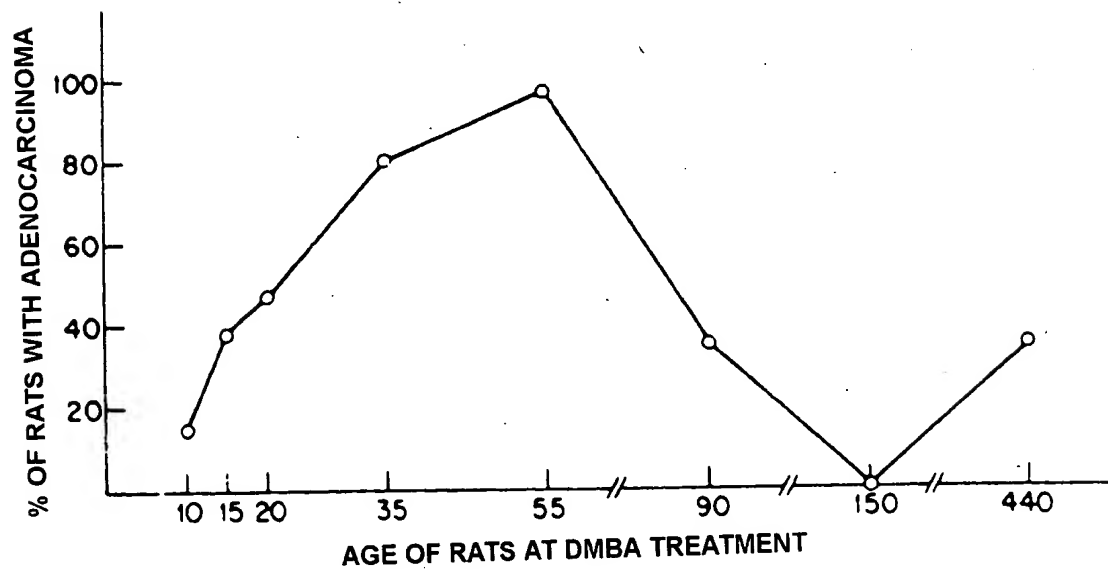
INCIDENCE OF MAMMARY ADENOCARCINOMA IN
RATS GIVEN DMBA AT DIFFERENT AGES

FIGURE 125

ANTI-HUMAN SHBG ANTIBODY IMMUNOPRECIPITATION OF THE ESTROGENIC ACTIVITY PRESENT IN CDE-HORSE SERUM WITH MTW9/PL2 CELLS
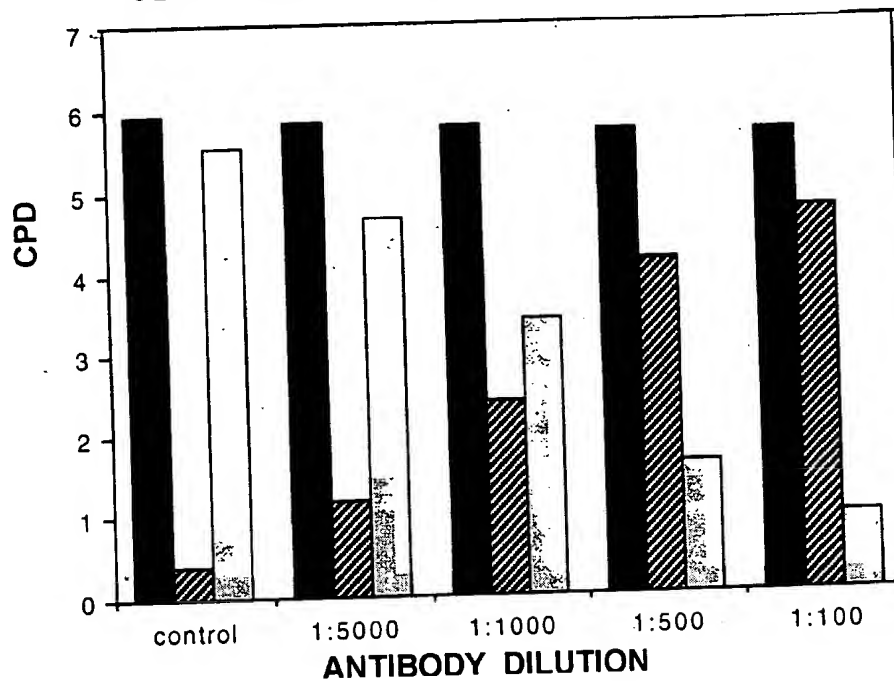
LEGEND: ■ = GROWTH IN 50% CDE WITH E$_2$

        ▨ = GROWTH IN 50% WITHOUT E$_2$

        ▣ = E$_2$ EFFECT
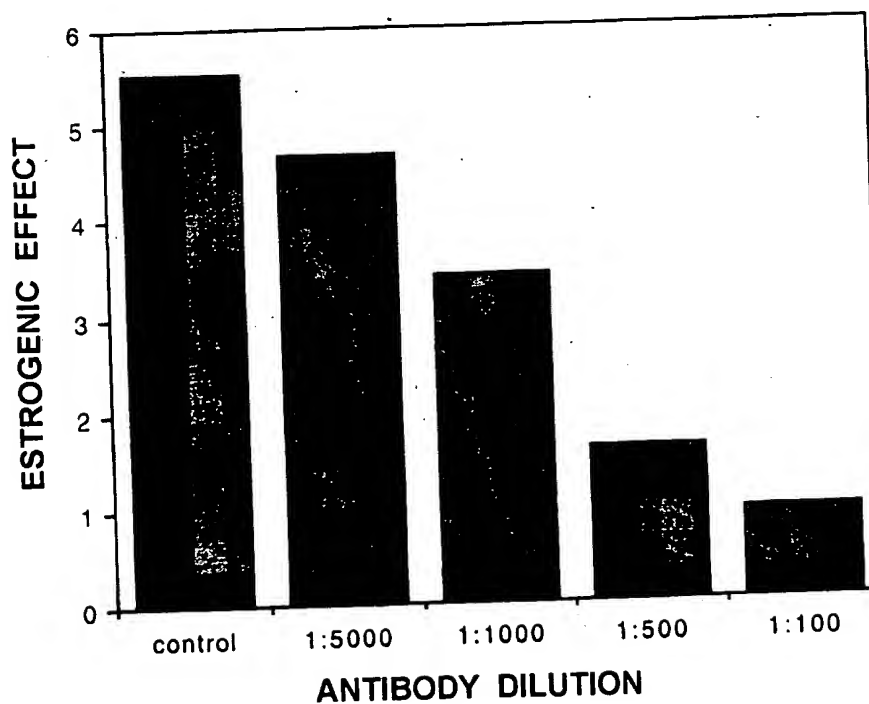
FIGURE 126

ANTI-HUMAN SHBG ANTIBODY IMMUNOPRECIPITATION OF THE ESTROGENIC ACTIVITY PRESENT IN CDE-RAT SERUM ASSAYED WITH MTW9/PL2 CELLS
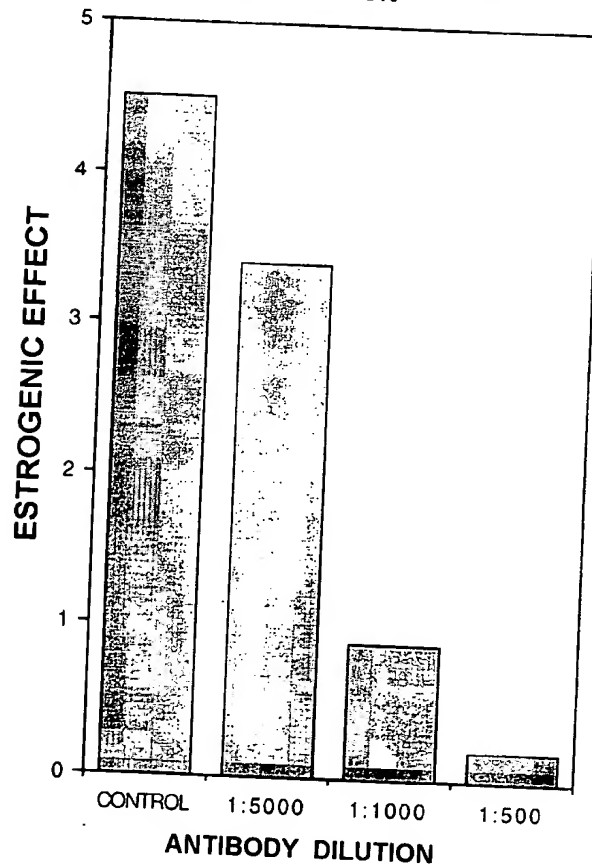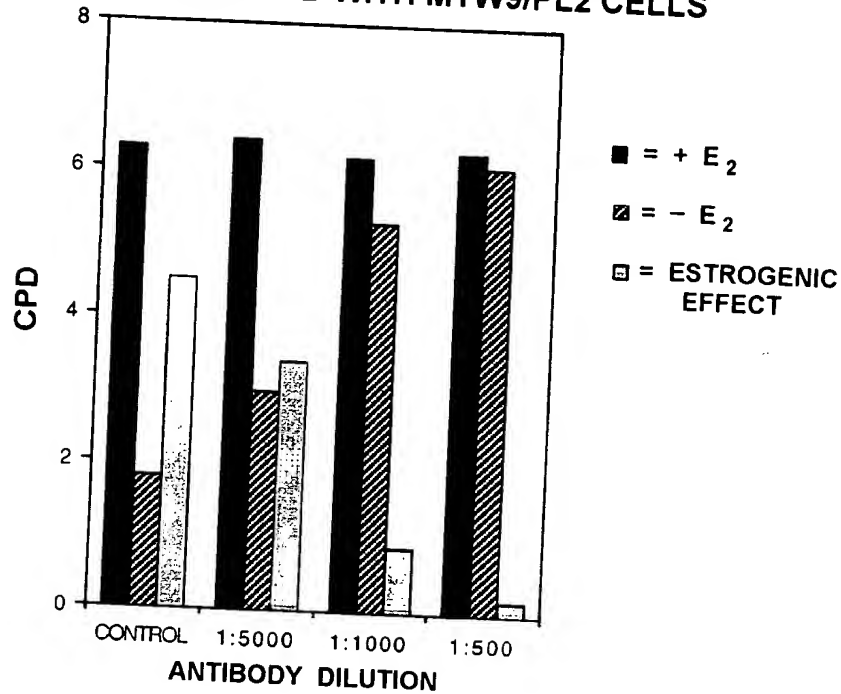
FIGURE 127

ANTI-HUMAN SHBG ANTIBODY IMMUNOPRECIPITATION OF THE LABELED STEROID HORMONE BINDING ACTIVITY PRESENT IN CDE-RAT SERUM
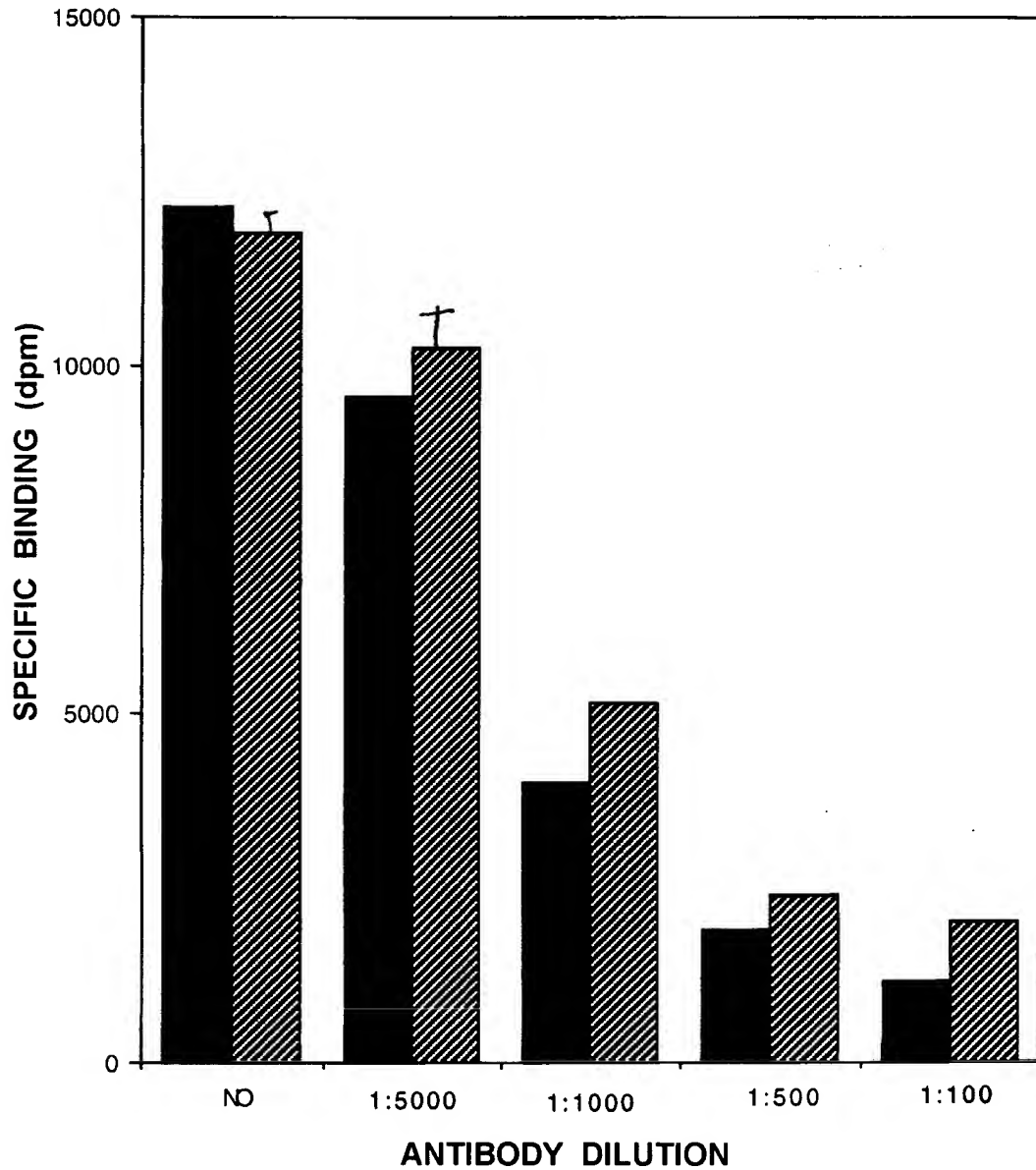
LEGEND: ■ = RAT

          ▨ = HORSE

FIGURE 128

WESTERN ANALYSIS AND DENSITOMETRY OF THE IMMUNOGLOBULIN LEVELS IN THE SERUM OF FEMALE RATS OF SPECIFIED AGE GROUPS
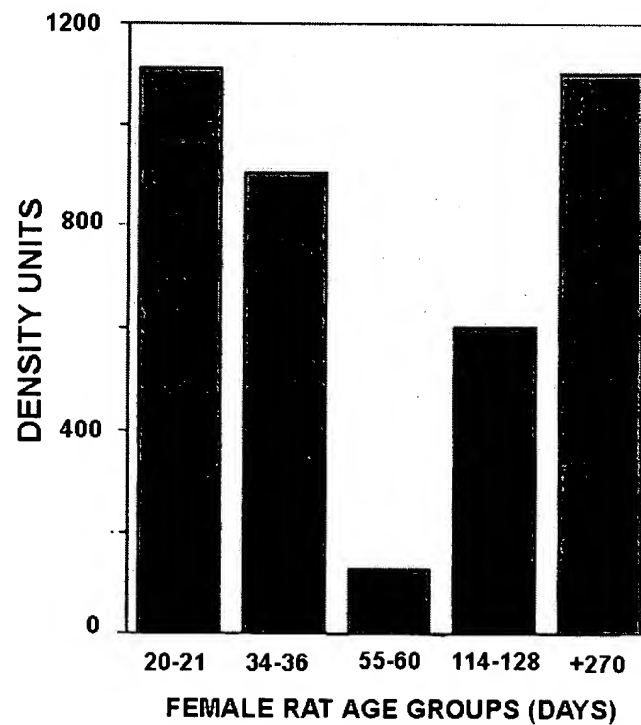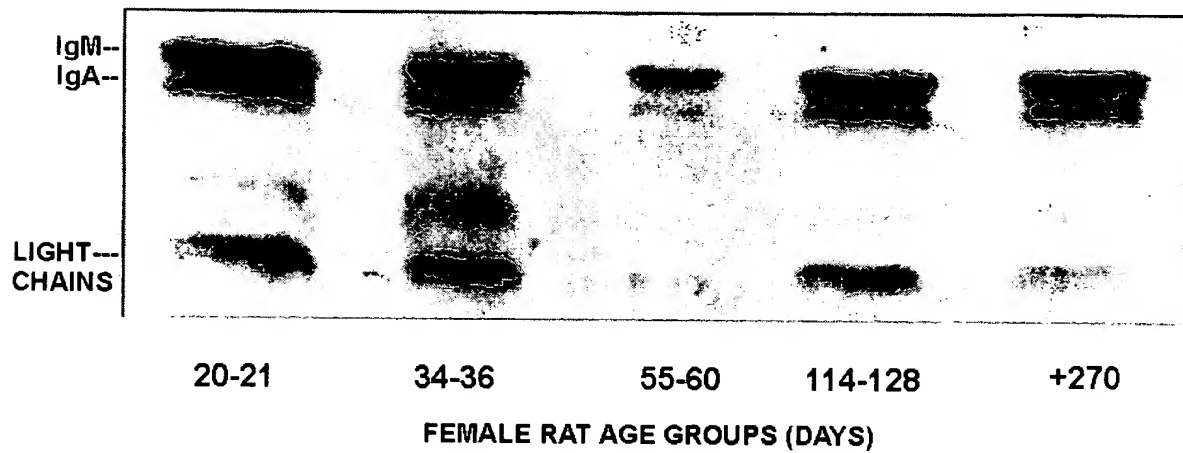
FIGURE 129

STRUCTURAL AND FUNCTIONAL ORGANIZATION

OF THE HUMAN ESTROGEN RECEPTOR-alpha

FIGURE 130

"BREAST CANCER" SEARCH - 31 "HITS"

"PIGR" (POLY-Ig RECEPTOR) SEARCH - 1 "HIT"

NOTE: THERE ARE NO BREAST CANCER "HITS" IN THE AREA OF THE POLY-Ig RECEPTOR ON CHROMOSOME 1

FIGURE 131

CHROMOSOME 1

BREAST
CANCER
LOCI

<-- PIGR

BREAST
CANCER
SEARCH

PIGR
SEARCH

FIGURE 132

CANCER AROUND THE WORLD, 1994-1997
DEATH RATES PER 100,000 (45 COUNTRIES)

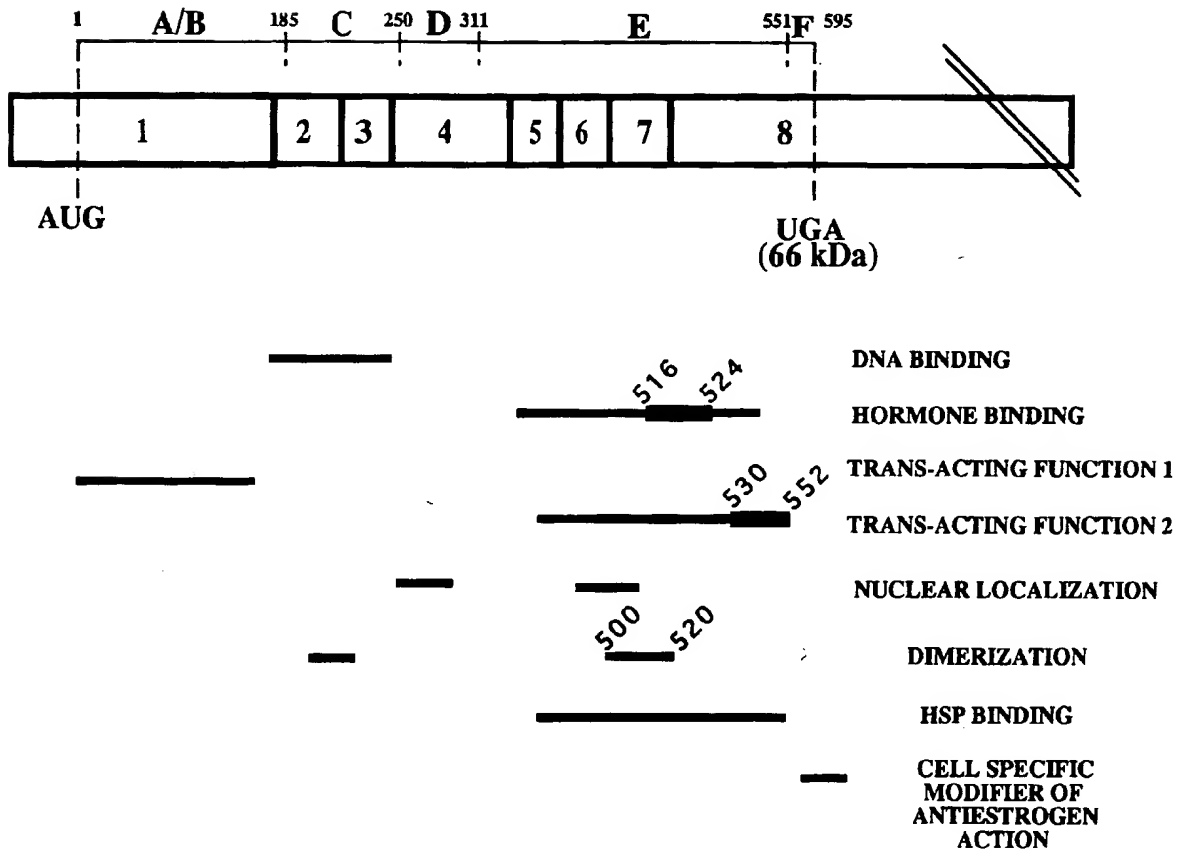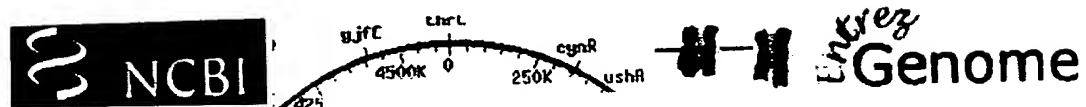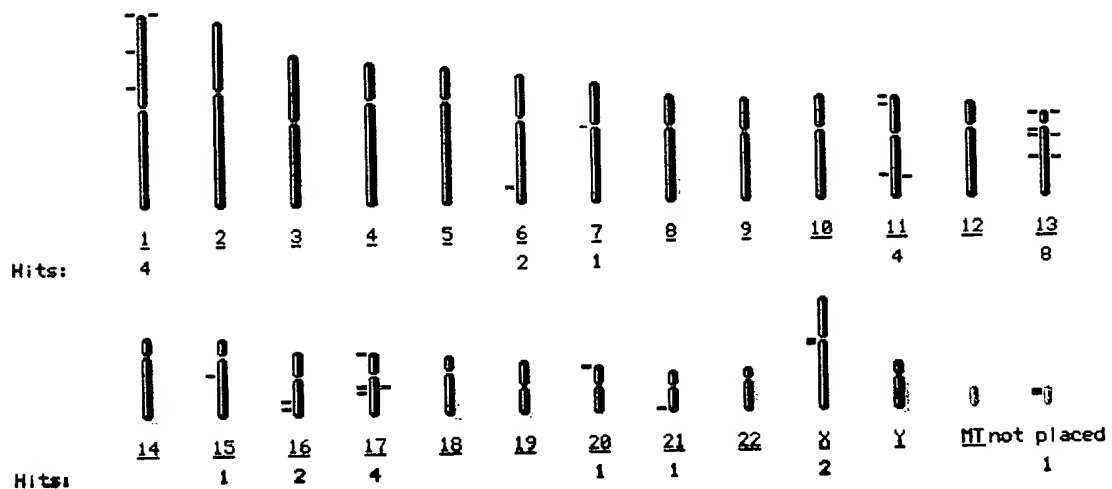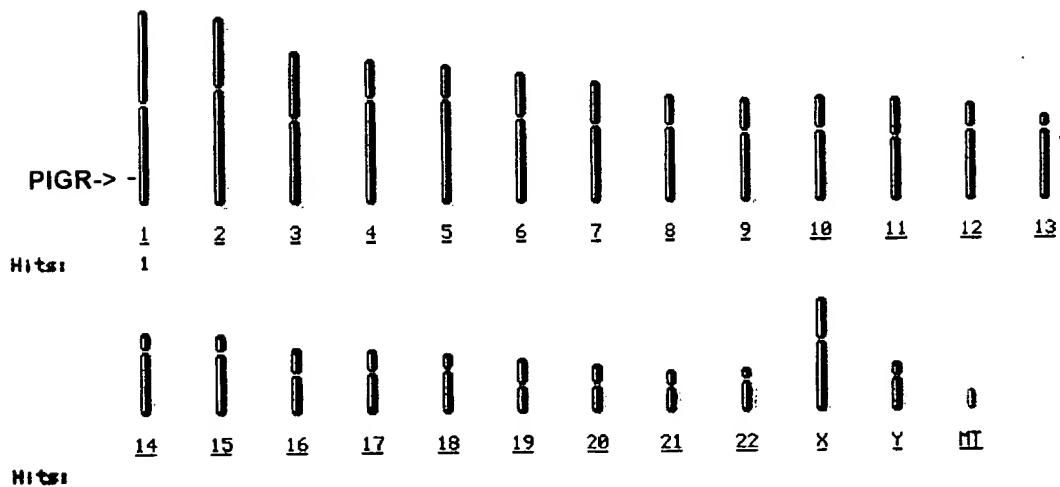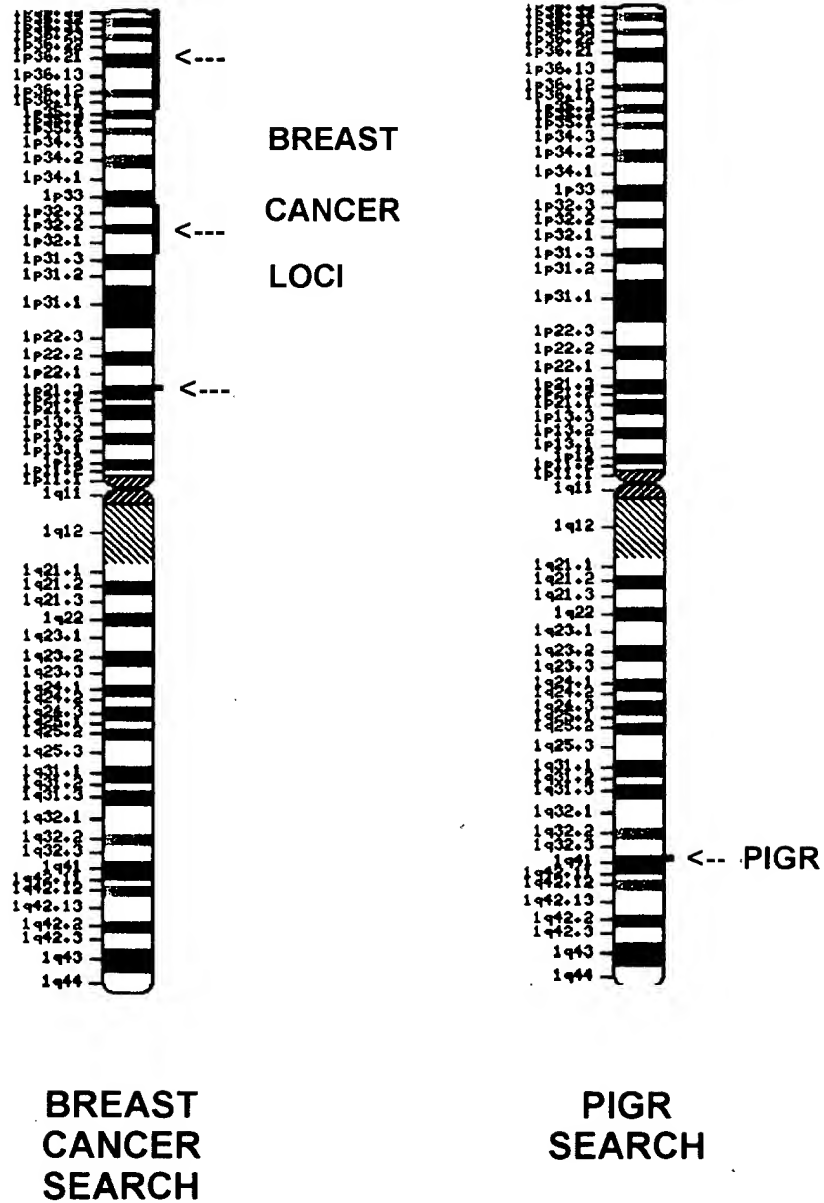| Country	Colon & Rectum		Breast	Prostate
	Male	Female	Female	Male
United States†	15.2 (27)	10.4 (23)	20.0 (14)	15.9 (20)
Australia‡	20.2 (10)	13.3 (10)	19.9 (15)	19.0 (9)
Austria†	21.7 (8)	12.2 (14)	20.9 (13)	16.9 (14)
Azerbaijan§	6.0 (41)	4.2 (43)	8.6 (42)	5.1 (41)
Bulgaria^	17.2 (20)	11.4 (19)	15.9 (31)	8.5 (34)
Canada‡	16.1 (26)	10.3 (25)	21.5 (10)	16.4 (17)
Chile^	7.0 (38)	6.7 (36)	12.1 (35)	16.0 (19)
China¶^	7.9 (36)	6.4 (37)	5.0 (44)	—
Colombia^	4.8 (44)	5.1 (40)	9.1 (40)	12.6 (28)
Croatia#	22.5 (6)	11.5 (18)	18.5 (20)	13.0 (25)
Cuba‡	9.4 (34)	11.3 (20)	14.9 (33)	20.8 (4)
Czech Republic§	34.3 (1)	17.3 (3)	21.1 (12)	16.0 (18)
Denmark§	22.7 (5)	15.6 (4)	27.6 (1)	19.9 (6)
Estonia§	18.1 (16)	12.2 (13)	18.5 (19)	12.8 (27)
Finland‡	12.1 (31)	8.5 (31)	16.8 (25)	17.6 (12)
France‡	16.6 (22)	9.6 (29)	19.6 (16)	15.8 (21)
Germany†	20.8 (9)	14.0 (7)	21.7 (8)	16.6 (16)
Greece§	8.0 (35)	6.2 (38)	16.2 (27)	9.3 (33)
Hungary^^	34.3 (2)	18.7 (2)	23.7 (6)	18.7 (11)
Ireland‡	22.5 (7)	13.3 (9)	26.1 (2)	18.8 (10)
Israel§	17.9 (18)	13.8 (8)	25.1 (4)	12.0 (30)
Japan**	17.1 (21)	9.9 (28)	7.7 (43)	5.1 (42)
Kazakhstan§	12.6 (30)	8.6 (30)	13.2 (34)	5.7 (39)
Kyrgyzstan§	6.9 (39)	4.5 (41)	10.6 (37)	4.3 (43)
Latvia‡	18.3 (12)	11.8 (15)	17.3 (24)	11.5 (31)
Lithuania§	18.2 (13)	11.7 (16)	18.7 (18)	15.2 (22)
Macedonia§	10.8 (33)	7.1 (34)	16.1 (30)	6.2 (38)
Mauritius§	6.0 (42)	3.8 (44)	9.0 (41)	7.7 (36)
Mexico‡	3.6 (45)	3.3 (45)	9.3 (39)	12.8 (26)
Netherlands‡	17.7 (19)	12.7 (11)	26.0 (3)	19.4 (8)
New Zealand^	26.4 (3)	19.1 (1)	22.9 (7)	19.8 (7)
Norway‡	20.0 (11)	14.7 (5)	19.4 (17)	23.2 (2)
Poland§	16.4 (23)	11.0 (22)	16.1 (29)	11.1 (32)
Portugal§	18.1 (15)	10.4 (24)	17.6 (22)	17.2 (13)
Rep. of Moldova‡	16.2 (25)	11.1 (21)	18.2 (21)	5.7 (40)
Romania§	11.3 (32)	7.9 (33)	15.7 (32)	8.3 (35)
Russian Fed.‡	18.2 (14)	12.6 (12)	16.1 (28)	7.2 (37)
Slovakia‡	14.6 (28)	6.8 (35)	—	12.2 (29)
Slovenia§	23.9 (4)	14.0 (6)	21.2 (11)	14.7 (23)
Spain‡	16.4 (24)	10.0 (27)	17.5 (23)	13.9 (24)
Sweden§	13.8 (29)	10.2 (26)	16.8 (26)	21.4 (3)
Trinidad & Tobago^	7.8 (37)	8.3 (32)	21.5 (9)	35.5 (1)
Turkmenistan^	6.2 (40)	4.4 (42)	9.5 (38)	1.4 (44)
United Kingdom†	18.0 (17)	11.6 (17)	24.5 (5)	16.6 (15)
Venezuela^	5.9 (43)	6.2 (39)	11.8 (36)	20.3 (5)

FIGURES IN PARENTHESES ARE ORDER OF RANK WITHIN SITE AND SEX GROUP

SOURCE: MORTALITY DATABASE 1994-97 WORLD HEALTH ORGANIZATION, 1999

FIGURE 133

A: TYPICAL CONCENTRATIONS OF IgG SUBCLASSES DURING CHILDHOOD
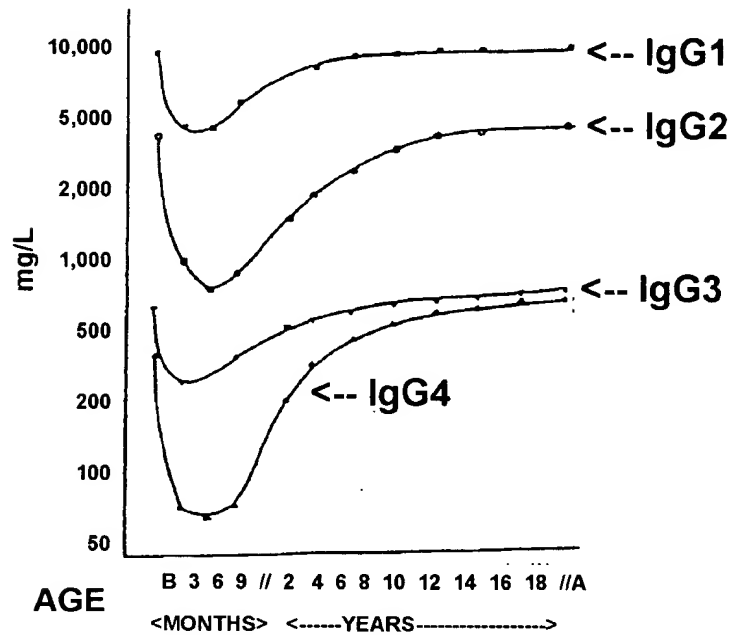
B: IMMUNOGLOBULIN CHANGES WITH AGE